VERIFICATION OF TRANSLATION

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declare as follows:

1.   That I am well acquainted with both the English and <u>Japanese</u> languages, and

2.   That the attached document is a true and correct translation made by me to the best of my knowledge and belief of:-

(a)   US Application No. 10/608,863

       Entitled: "METHODS OF TESTING FOR ALLERGIC DISEASES AND THERAPEUTIC AGENTS FOR TREATING SAME"

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METHODS OF TESTING FOR ALLERGIC DISEASES AND THERAPEUTIC AGENTS FOR TREATING SAME

FIELD OF THE INVENTION

5   The present invention relates to a method of testing for allergic diseases and a method for screening candidate compounds as therapeutic agents for an allergic disease using the expression of NOR-1 gene related to allergic diseases as an index. The present invention is also directed towards pharmaceutical agents for treating
10   allergic diseases.

BACKGROUND OF THE INVENTION

Allergic diseases such as atopic dermatitis are considered multifactorial diseases. These diseases are caused by the
15   interaction of many different genes whose expression is independently influenced by multiple environmental factors. Thus, determination of specific genes causing a specific disease has been extremely difficult for allergic diseases.

Furthermore, the expression of mutated or defective genes or
20   overexpression or reduced expression of specific genes is assumed involved in allergic diseases. Therefore, to elucidate the role of gene expression in diseases, it is necessary to understand how a gene is involved at the onset of a disease and how the expression of the gene is altered by external stimulants such as drugs.
25   Recent developments in gene expression analysis techniques have enabled analysis and comparison of gene expression of many clinical samples. Among these methods, the differential display (DD) method is useful. Liang and Pardee originally developed this method in 1992 (Science, 1992, 257: 967-971). According to this method, several
30   tens or more different samples can be screened at one time to detect genes whose expressions are different among the samples. Important information to reveal the causative gene of a disease is expected to be obtained by examining genes with mutations or genes whose expression changes depending on the time and environment. Such genes
35   include those whose expression is influenced by environmental factors.

Medical examination by interview with the patient, confirmation
of family history and anamnesis of the patient are generally important
for recent diagnosis of allergic diseases. Furthermore, methods of
diagnosing allergies based on information that is more objective
include a method in which patient's blood sample is tested and a method
of observing patient's immune response to allergen. Examples of the
former method are the allergen-specific IgE measurement, leukocyte
histamine release test and lymphocyte blast transformation test. The
presence of allergen-specific IgE verifies the allergic reaction
against the allergen. However, allergen-specific IgE is not always
detected in every patient. Furthermore, the principle of IgE assay
requires performing tests for all of the allergens necessary for
diagnosis. The leukocyte histamine release test and lymphocyte blast
transformation test are methods for observing the reaction of the
immune system toward a specific allergen *in vitro*. These methods
require complex operation.

Another known method for allergy diagnosis is based on the
immune response observed at the time when a patient contacts an
allergen (latter method). Such tests include the prick test, scratch
test, patch test, intradermal reaction and induction test. These
tests allow direct diagnosis of a patient's allergic reaction, but
are highly invasive tests because the patients are actually exposed
to allergen.

In addition, regardless of the allergen types, methods to
confirm the involvement of an allergic reaction are also attempted.
For example, a high serum IgE titer indicates the occurrence of
allergic reaction in a patient. The serum IgE titer corresponds to
the total amount of allergen-specific IgE. Though it is easy to
determine the total amount of IgE regardless of the type of allergen,
IgE titer may be reduced in some patients, for example, those with
non-atopic bronchitis.

The number of eosinophils and eosinophil cationic protein (ECP)
levels are diagnostic items for delayed-type reaction following Type
I allergy and allergic inflammatory reaction. The number of
eosinophils is considered to reflect the progress of allergic symptoms.
ECP, a protein contained in eosinophil granules, is also strongly

activated in patients with an asthma attack. Indeed, symptoms of allergies can be identified using these diagnostic items. However, only limited range of values can be used as a diagnostic index.

Therefore, diagnostic indices, regardless of the type of allergen, useful in comprehending pathological conditions of allergic disease patients and for determining the treatment regimen for the disease have been greatly needed in the art. Markers for allergic disease that are not only less harmful to patients but also capable of readily providing information required for diagnosis would be of great use. Identification of genes associated with allergic diseases enables tests for allergic diseases via the use of the expression of the genes as indexes. Furthermore, elucidation of the function of proteins encoded by the genes at the cell level is expected to promote, based on findings relating to their function, development of therapeutic methods and pharmaceutical agents for treating allergic diseases.

SUMMARY OF THE INVENTION

The present invention was achieved in the light of the above context, and the objective of the invention is to identify a gene associated with allergic diseases. Furthermore, another objective of the present invention is to provide a method of testing for allergic diseases and a method of screening for candidate compounds for a therapeutic agent for an allergic disease, both using the expression of the gene as an index, as well as pharmaceutical agents for treating allergic diseases.

Based on a previously established procedure, the "fluorescent differential display method (Fluorescent DD method)" (T. Ito et al. 1994, FEBS Lett. 351: 231-236), the present inventors developed a new DD system capable of analyzing leukocyte RNA samples prepared from multiple human blood samples (Japanese Patent Application No. Hei 11-120489). Utilizing the DD system, the present inventors tried to identify genes whose expression level is altered in an allergic disease-specific manner.

Specifically, first, the present inventors compared several parameters relating to allergic symptoms in patients with atopic

dermatitis (a typical allergic disease), between the exacerbation stage and the remission stage of dermatitis conditions. As a result, decrease of eosinophils in the remission stage was observed in some patients. Since eosinophils are generally used as a typical clinical index for atopic dermatitis, the present inventors focused on this aspect. Furthermore, they considered that a gene directly involved in atopic dermatitis may be isolated by isolating a gene whose expression level in eosinophils differs between the exacerbation stage and the remission stage of a patient.

Therefore, eosinophils were collected from several subjects in the exacerbation stage and the remission stage of atopic dermatitis, and genes whose expression level alters in the eosinophils were screened using the aforementioned system. As a result, the present inventors succeeded in isolating sequence "2250-01" that showed a significantly higher expression level in patients that were observed to show a decrease of eosinophils in the remission stage. Genomic database analysis revealed this sequence as the intronic region of a nuclear orphan receptor called NOR-1(MINOR). Therefore, the expression of the reported exon sequence of NOR-1(MINOR) was determined and, like the expression of the intronic region, found to be induced in the remission stage of subjects who clearly showed a decrease of eosinophils. No relation of the NOR-1 gene to allergic diseases has been reported so far.

The elevation of a gene that is suggested to have apoptotic character observed in peripheral blood eosinophils during the remission stage during treatment of atopic dermatitis corresponds well with the decrease in the number of peripheral blood eosinophils. Therefore, the induction of the NOR-1 gene expression would be correlated with therapeutic effect.

Using the expression level of the NOR-1 gene of this invention as an index, allergic diseases may be tested.

Furthermore, NOR-1 receptor is an orphan receptor and hitherto, neither the *native* ligand nor activator of the receptor had been found. The present inventors developed a high-throughput system for searching ligands and using this system, succeeded in obtaining a compound that may have a function to activate NOR-1 transcription.

This compound is a prostaglandin (PGA derivative) comprising a cyclopentenone structure and may be the native ligand of the NOR-1 receptor. Furthermore, experiments using mutants wherein the ligand-binding domain (LBD) region of the receptor is deleted indicated that the prostaglandin derivative actually functions by acting on the LBD region of the receptor. Moreover, experiments utilizing BIAcor demonstrated the binding of the PGA derivative to NOR-1.

Thus, the present inventors found that screening of candidate compounds for a therapeutic agent for an allergic disease is possible, and the PGA derivative is a ligand activator of NOR-1.

Furthermore, using a pharmacophore model, the present inventors simulated the binding site of the PGA derivative to the NOR-1 ligand binding domain, and based on information on structure-activity relationship for the reporter system of the PGA derivative, compounds other than the PGA derivative that match with the binding pocket were selected from the database. These compounds are expected to function as ligands of the NOR-1 receptor.

Therapeutic effects on allergic diseases are expected for compounds that induce expression of the NOR-1 gene, or compounds that bind to the NOR-1 receptor and promote its transcription activity (for example, ligand activators).

Furthermore, the present inventors found for the first time that the expression of NOR-1 in cultured peripheral blood eosinophils is dramatically induced due to apoptosis stimulation of the cell with anti-CD30 antibody that has an agonist activity towards eosinophil CD30. Therefore, a therapeutic agent for allergic diseases is provided, which agent has the mechanism of increasing the expression of the NOR-1 gene by eosinophil CD30 ligand stimulation and inducing apoptosis of eosinophils by regulating the expression of downstream genes of Nor1.

The present inventors further succeeded in engineering a transgenic mouse wherein the expression of the human NOR-1 gene is induced through an actin promoter. This mouse is extremely useful as an animal model for analyzing allergic diseases mediated via NOR-1.

The present invention relates to a method of testing for

allergic diseases and a method for screening candidate compounds as therapeutic agents for an allergic disease. These methods are performed using, as an index, the expression of the NOR-1 gene that shows high expression during allergic diseases, particularly in the remission stage associated with the decrease of eosinophils. The present invention also relates to pharmaceutical agents for treating allergic diseases. Specifically, the present invention provides:

[1] a method of testing for an allergic disease, said method comprising the steps of:

(a) measuring the expression level of NOR-1 receptor protein or a gene encoding the protein in eosinophil cells of a test subject; and

(b) comparing the expression level with that in eosinophil cells of a healthy subject;

[2] the testing method of [1], wherein the gene expression level is measured by cDNA PCR;

[3] the testing method of [1] or [2], wherein the allergic disease is atopic dermatitis;

[4] a reagent for testing for an allergic disease, said reagent comprising an oligonucleotide that has a length of at least 15 nucleotides and comprises a nucleotide sequence complementary to a polynucleotide encoding an NOR-1 receptor protein or to its complementary strand;

[5] a method of detecting the influence of a candidate compound on the expression level of a polynucleotide of (a) or (b) below, wherein said method comprises the steps of:

(1) contacting the candidate compound with a cell that expresses a polynucleotide of (a) or (b):

(a) a polynucleotide encoding an NOR-1 receptor protein; and

(b) a polynucleotide encoding a protein whose expression in eosinophils increases in association with the decrease of eosinophils in the remission stage of atopic dermatitis, wherein said polynucleotide hybridizes under stringent conditions with a polynucleotide encoding an NOR-1 receptor protein; and

(2) measuring the expression level of the polynucleotide (a) or (b);

[6] the method of [5], wherein the cell is a leukocyte cell line;

[7] a method of detecting the influence of a candidate compound on the expression level of a polynucleotide of (a) or (b) below, wherein said method comprises the steps of:

     (1) administering the candidate compound to a test animal; and

     (2) measuring, in the eosinophil cells of the test animal, the expression intensity of a polynucleotide of (a) or (b):

     (a) a polynucleotide encoding an NOR-1 receptor protein; and

     (b) a polynucleotide encoding a protein whose expression in eosinophils increases in association with the decrease of eosinophils in the remission stage of atopic dermatitis, wherein said polynucleotide hybridizes under stringent conditions with a polynucleotide encoding an NOR-1 receptor protein;

[8] a method of screening for a compound that increases the expression level of the polynucleotide (a) or (b), wherein said method comprises the steps of detecting the influence on the expression level by the method of any one of [5] to [7], and selecting a compound that increases the expression level compared to a control;

[9] a method of detecting the influence of a candidate compound on the expression level of a polynucleotide encoding an NOR-1 receptor protein, wherein said method comprises the steps of:

     (1) contacting a candidate compound with a cell or cell extract containing a DNA having a structure such that the transcription regulatory region of a gene encoding an NOR-1 receptor protein and a reporter gene are operably linked; and

     (2) measuring the activity of the reporter gene;

[10] a method of screening for a candidate compound that increases the expression level of a gene encoding an NOR-1 receptor protein, wherein said method comprises the steps of detecting the influence of a compound on the activity by the method of [9], and selecting a compound that increases the activity compared to a control;

[11] a method of screening for a candidate compound for a therapeutic agent for an allergic disease, wherein said method comprises the steps of:

     (1) contacting a test compound with an NOR-1 receptor protein;

     (2) measuring the binding activity between the test compound

and the NOR-1 receptor protein; and

(3) selecting a compound that binds to the NOR-1 receptor protein;

[12] a method of screening for a candidate compound for a therapeutic agent for an allergic disease, wherein said method comprises the steps of:

(1) providing cells transfected with (a) a DNA that can express a fusion protein of an NOR-1 receptor protein or its ligand binding domain and a transcription regulatory region binding protein, and (b) a DNA having a structure such that a reporter gene is operably linked downstream of a DNA sequence to which the transcription regulatory region binding protein binds;

(2) contacting the cell with a test compound;

(3) measuring the activity of the reporter gene; and

(4) selecting a compound that changes the activity;

[13] a therapeutic agent for an allergic disease, said agent comprising, as an active ingredient, a compound obtainable by the screening method of any one of [10] to [12];

[14] a therapeutic agent for an allergic disease, said agent comprising, as an active ingredient, a prostaglandin having a cyclopentenone structure, which is obtainable by the screening method of any one of [10] to [12];

[15] a therapeutic agent for an allergic disease, said agent comprising, as an active ingredient, a ligand of an NOR-1 receptor;

[16] the therapeutic agent for an allergic disease of [15], wherein the ligand of an NOR-1 receptor is a prostaglandin having a cyclopentenone structure;

[17] the therapeutic agent for an allergic disease of [16], wherein the prostaglandin having a cyclopentenone structure is selected from the group consisting of prostaglandin A_2, prostaglandin A_1, 16,16-dimethyl prostaglandin A_2, 15(R)-15-methyl prostaglandin A_2, 16-phenoxy tetranor prostaglandin A_2, 17-phenyl trinor prostaglandin A_2, 15-deoxy-delta 12,14-prostaglandin J_2, and 8-iso prostaglandin A_1;

[18] the therapeutic agent for an allergic disease of [15], wherein the ligand of an NOR-1 receptor is any one of the compounds listed

in Tables 14 to 58;

[19] the therapeutic agent for an allergic disease of any one of [13] to [18], wherein the allergic disease is atopic dermatitis;

[20] an animal model for an allergic disease, wherein the animal model is a transgenic non-human vertebrate wherein the expression intensity of polynucleotide (a) or (b) below is decreased in eosinophil cells:

      (a) a polynucleotide encoding an NOR-1 receptor protein; and

      (b) a polynucleotide encoding a protein whose expression in eosinophils increases in association with the decrease of eosinophils in the remission stage of atopic dermatitis, wherein said polynucleotide hybridizes under stringent conditions with a polynucleotide encoding an NOR-1 receptor protein;

[21] the animal model of [20], wherein the transgenic animal is a knockout animal;

[22] a method of inducing apoptosis of a cell, said method comprising activation of an NOR-1 receptor protein in the cell;

[23] the apoptosis induction method of [22], which comprises the step of contacting a cell with a compound or a prostaglandin having a cyclopentenone structure, which is obtainable by the screening method of any one of [10] to [12];

[24] the apoptosis induction method of [22] or [23], wherein said cell is an eosinophil cell;

[25] an apoptosis inducing agent, which comprises a compound or a prostaglandin having a cyclopentenone structure, which is obtainable by the screening method of any one of [10] to [12];

[26] an apoptosis-inducing agent comprising a ligand of an NOR-1 receptor as an active ingredient;

[27] the apoptosis-inducing agent of [26], wherein the ligand of an NOR-1 receptor is a prostaglandin having a cyclopentenone structure;

[28] the apoptosis-inducing agent of [27], wherein the prostaglandin having a cyclopentenone structure is selected from the group consisting of prostaglandin A_2, prostaglandin A_1, 16,16-dimethyl prostaglandin A_2, 15(R)-15-methyl prostaglandin A_2, 16-phenoxy tetranor prostaglandin A_2, 17-phenyl trinor prostaglandin A_2, 15-deoxy-delta 12,14-prostaglandin J_2, and 8-iso prostaglandin A_1;

[29] the apoptosis-inducing agent of [26], wherein the ligand of an

NOR-1 receptor is any one of the compounds listed in Tables 14 to 58; and

[30] a NOR-1 gene expression-inducing agent, which comprises a ligand of an eosinophil CD30 receptor.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 shows the 2250-01 expression levels (copy/ng RNA), corrected for β-actin, in the exacerbation stage and the remission stage of atopic dermatitis patients (patient numbers 1 to 7).

Fig. 2 shows the expression levels of NOR-1 gene determined using the open reading frame (ORF) site of the NOR-1 gene, only in patients whose number of eosinophils decreased with the transition from the exacerbation stage to the remission stage.

Fig. 3 shows a schematic illustration of a ligand searching system for the NOR-1 receptor constructed by the present inventors. A full-length gene or the ligand-binding site of NOR-1 is inserted into X, and the full-length gene of the retinoic acid X receptor (RXR)α is inserted into Y. These constructs are transfected into NIH3T3 cells, and the activity of induced luciferase is measured.

Fig. 4 shows a schematic illustration of the structure of the NOR-1 receptor protein.

Fig. 5 shows the NOR-1 transcriptional activating function of prostaglandin A_2 and prostaglandin A_1 used in the system of Fig. 3.

Fig. 6 shows the structure of 15(R)-15-methyl-prostaglandin A_2 in a gamma model wherein the binding position of the PGA derivative to the NOR-1 ligand-binding domain is simulated using the pharmacophore model.

Fig. 7 shows a graph demonstrating the decrease of the transcriptional activity of prostaglandin A_2 due to use of the LBD deletion mutant of NOR-1. ΔLBD denotes the deletion mutant.

Fig. 8 shows diagrams that show the state, revealed with BIAcor S51, wherein PGA1 and PGA2 bind to the LBD of NOR-1. Glutathione S-transferase (GST) was used as a comparison control and 13,14-Dihydro-15-keto-PGA2 as a negative control.

Fig. 9 shows graphs indicating the result of expression induction of NOR-1 due to apoptosis stimulation of peripheral blood

eosinophils using anti-CD30 antibody or anti-Fas antibody. Values collected for beta-actin and GAPDH are shown.

Fig. 10 shows a graph indicating the apoptosis induction effect due to the treatment of eosinophil-specific cell line, AML14.3D10,
5   with anti-CD30 antibody or anti-Fas antibody.

Fig. 11 shows a graph indicating the expression induction of NOR-1 due to the treatment of eosinophil-specific cell line, AML14.3D10, with anti-CD30 antibody or anti-Fas antibody.

Fig. 12 shows a working hypothesis for an allergic disease
10   treatment via eosinophil cell death caused by nuclear receptor Nur subfamily including Nor1.

Fig 13 shows a photograph of NOR-1 transgenic mouse and wild-type mouse. Characteristics such as, a decrease in the body weight to half, thymic atrophy and splenic atrophy were observed in
15   the transgenic mouse.

DETAILED DESCRIPTION OF THE INVENTION

Through the comparison of the exacerbation stage and remission stage of an atopic dermatitis patient, the present inventors found
20   that the expression level of the NOR-1(MINOR) gene (in the present specification, it may be simply referred to as "NOR-1") increases in eosinophils of patients in the remission stage associated with the decrease of eosinophils. Therefore, allergic diseases can be tested in subjects using the expression level of the NOR-1 gene as
25   an index.

The present invention provides a method of testing for allergic diseases, which comprises measuring the expression level of the NOR-1 gene.

A preferred embodiment of the method of the present invention
30   comprises the steps of:

(a) measuring the expression level of a gene encoding the NOR-1 receptor protein in eosinophils of a test subject; and

(b) comparing the measured expression level to the expression level of the gene in eosinophil cells of a healthy subject.

35   NOR-1(MINOR) receptor is a γ-type receptor of the nuclear orphan receptors that has various names and constitute three subfamilies

as shown in Table 1, and is mainly called NOR-1 regardless of the species.

Table 1

	Human	Mouse	Rat
α	NAK-1(TR3)	nur77	NGFI-B
β	TINUR/NOT	Nurr1	RNR-1
γ	MINOR/CHN	TEC	NOR-1

5

   Information relating to the amino acid sequences of these NOR-1 (MINOR) receptor proteins or the nucleotide sequences of genes encoding the proteins can be readily obtained from various gene databases known to those skilled in the art. Specifically, the
10   nucleotide sequence of a gene encoding the human NOR-1 receptor protein (NOR-1 gene) is shown in SEQ ID NO: 1, and the amino acid sequence of the NOR-1 receptor protein is shown in SEQ ID NO: 2.

   Herein, the general phrase "allergic disease" is refers to diseases involving allergic reactions. More specifically, it is
15   defined as a disease for which an allergen is identified, a strong correlation between the exposure to the allergen and the onset of the pathological change is demonstrated, and the pathological change has been proven to have an immunological mechanism. Herein, an immunological mechanism means that leukocytes show an immune response
20   to allergen stimulation. Examples of allergens include mite antigen and pollen antigen.

   Representative allergic diseases include bronchial asthma, allergic rhinitis, atopic dermatitis, pollen allergy and insect allergy. Allergic diathesis is a genetic factor that is inherited
25   from allergic parents to their children. Familial allergic diseases are also called atopic diseases, and their causative factor that is inherited is the atopic diathesis. The term "atopic dermatitis" is a general term for atopic diseases with dermatitis among atopic diseases.

30   The tests for allergic diseases of the present invention include following tests. A test for determining whether a subject is affected with an allergic disease or not, a test for determining whether a

subject has a trait of being easily affected by an allergic disease or not, and a test for assessing whether the allergic symptom is improving or not. The NOR-1 gene of this invention showed increased expression level especially in the eosinophils of atopic dermatitis
5   patients in the remission stage associated with the decrease of eosinophils. Since eosinophils are a representative clinical marker for atopic dermatitis, a clinical marker associated with its decrease is useful for assessing therapeutic effects. More specifically, increased expression of the NOR-1 gene indicates that the allergic
10   disease is improving with the decrease of eosinophils.

The severity of atopic dermatitis and the number of eosinophils are correlated. In fact, actively decreasing the number of eosinophils may lead to curing the disease. In relation to the measurement of these genes that are specifically induced in
15   eosinophils accompanied with the decrease of its numbers, when methods or substances that actively induce these genes from outside the cell are found, they may lead to novel therapeutic methods for atopic dermatitis and diagnostic methods for evaluating the therapeutic methods.

20   Herein, the expression level of the NOR-1 gene includes transcription of the gene to mRNA as well as translation into protein. Therefore, a method of testing for an allergic disease according to the present invention is performed by comparing the expression intensity of mRNA corresponding to the gene, or the expression level
25   of protein encoded by the gene.

Measurement of the expression level of the NOR-1 gene in a method of testing for allergic diseases of the present invention may be conducted according to gene analytical methods known to those skilled in the art. More specifically, for example, a hybridization
30   technique with a nucleic acid that hybridizes to the NOR-1 gene as a probe, a gene amplification technique with a DNA hybridizing to the gene of this invention as a primer, or such can be utilized.

A primer or probe that can be used as a reagent for testing for an allergic disease according to the present invention includes: a
35   polynucleotide comprising at least 15 nucleotides that are complementary to the nucleotide sequence of SEQ ID NO: 1 or the

complementary strand thereof. Herein, the term "complementary strand" refers to one strand of a double stranded DNA, which is composed of A:T (or A:U for RNA) and G:C base pairs, to the other strand. In addition, "complementary" means not only those completely

5   complementary to a region of at least 15 continuous nucleotides, but also having a homology of at least 70%, preferably at least 80%, more preferably 90%, and even more preferably 95% or higher. The degree of homology between nucleotide sequences can be determined by the algorithm such as BLASTN.

10   Such polynucleotides are useful as probes to detect and isolate the polynucleotide encoding the protein according to the present invention, or as primers to amplify the polynucleotide according to the present invention. When used as a primer, those polynucleotides have a chain length of usually 15 bp to 100 bp, preferably 15 bp to

15   35 bp. When used as a probe, DNAs comprising the whole sequence of the polynucleotide according to the present invention, or its partial sequence that contains at least 15-bp, are used. When used as a primer, its 3' region must be complementary to the polynucleotide of the present invention, while the 5' region can be linked to a restriction

20   enzyme-recognition sequence or tag.

The "polynucleotides" of the present invention may be either DNA or RNA. These polynucleotides may be either synthetic (isolated) or naturally occurring. In addition, DNA used as a probe for hybridization is usually labeled. Examples of labeling methods are

25   described below. Herein, the term "oligonucleotide" refers to polynucleotides with relatively low degree of polymerization. Oligonucleotides are included in polynucleotides. The labeling methods are as follows:

·nick translation labeling using DNA polymerase I;

30   ·end labeling using polynucleotide kinase;

·fill-in end labeling using Klenow fragment (Berger, SL, Kimmel, AR. (1987) Guide to Molecular Cloning Techniques, Method in Enzymology, Academic Press; Hames, BD, Higgins, SJ (1985) Genes Probes: A Practical Approach. IRL Press; Sambrook, J, Fritsch, EF, Maniatis,

35   T. (1989) Molecular Cloning: a Laboratory Manual, 2nd Edn. Cold Spring Harbor Laboratory Press);

·transcription labeling using RNA polymerase (Melton, DA, Krieg, PA, Rebagkiati, MR, Maniatis, T, Zinn, K, Green, MR. (1984) Nucleic Acid Res., 12, 7035-7056); and

·non-radioisotopic labeling of DNA by incorporating modified
5 nucleotides (Kricka, LJ. (1992) Nonisotopic DNA Probing Techniques. Academic Press).

For testing for allergic diseases using hybridization techniques, for example, Northern hybridization, dot blot hybridization or DNA microarray technique may be used. Furthermore,
10 gene amplification techniques, such as RT-PCR method may be used. By using the PCR amplification monitoring method during the gene amplification step in RT-PCR, one can achieve quantitative analysis for the gene expression of the present invention.

In the PCR gene amplification monitoring method, the detection
15 target (DNA or reverse transcript of RNA) is hybridized to probes that are dual-labeled at both ends with different fluorescent dyes whose fluorescence cancels each other out. As the PCR proceeds and the Taq polymerase degrades the probe due to its 5'-3' exonuclease activity, the two fluorescent dyes become distant from each other
20 and the fluorescence is detected. The fluorescence is detected in real time. By simultaneously measuring a standard sample in which the copy number of the target is known, it is possible to determine the copy number of the target in the subject sample with the cycle number where PCR amplification is linear (Holland, P. M. et al., 1991,
25 Proc. Natl. Acad. Sci. USA 88: 7276-7280; Livak, K. J. et al., 1995, PCR Methods and Applications 4(6): 357-362; Heid, C. A. et al., 1996, Genome Research 6: 986-994; Gibson, E. M. U. et al., 1996, Genome Research 6: 995-1001). For example, for the PCR amplification monitoring method ABI PRISM7700 (PE Biosystems) may be used.

30 The method of testing for allergic diseases of the present invention can also be carried out by detecting a protein encoded by the NOR-1 gene. For example, test methods such as Western blotting, immunoprecipitation and ELISA using an antibody that binds to a protein encoded by this gene may be employed.

35 Antibodies that bind to the NOR-1 protein used in the detection may be produced by techniques well known to those skilled in the art.

Antibodies used in the present invention may be polyclonal or monoclonal (Milstein, C. et al., 1983, Nature 305 (5934): 537-40). For example, polyclonal antibodies against the protein of the present invention may be produced by collecting blood from mammals sensitized with an antigen, and separating the serum from this blood using known methods. As polyclonal antibodies, serum containing polyclonal antibodies may be used. If needed, a fraction containing polyclonal antibodies can be further isolated from this serum. Alternatively, monoclonal antibodies may be obtained by isolating immune cells from mammals sensitized with an antigen; fusing these cells with myeloma cells, and such; cloning the obtained hybridomas; and collecting the antibodies from the culture as the monoclonal antibody.

To detect the NOR-1 protein, these antibodies may be appropriately labeled. Alternatively, instead of labeling the antibodies, a substance that specifically binds to antibodies, for example, protein A or protein G, may be labeled to indirectly detect the protein. Specifically, one example of indirect detection method is ELISA.

A protein or its partial peptide used as an antigen may be obtained, by inserting the NOR-1 gene or its portion into an expression vector, introducing the vector into an appropriate host cell to produce a transformant, culturing the transformant to express the recombinant protein, and purifying the expressed recombinant protein from the culture or the culture supernatant. Alternatively, oligonucleotides consisting of a partial amino acid sequence of the amino acid encoded by the NOR-1 gene can be chemically synthesized to be used as the immunogen.

In this invention, it is preferred that eosinophils of a test subject are used as the sample. Eosinophils can be prepared by conventional methods from peripheral blood. Specifically, leukocytes are isolated, for example, by fractionating heparinized blood by centrifugation. Next, granulocytes are fractionated, for example, by Ficoll centrifugation of leukocytes, and furthermore eosinophils can be isolated, by depletion of neutrophils using the CD16 antibody. A sample for immunological assays of the aforementioned protein can be obtained by disrupting the isolated

eosinophils to produce a lysate. Alternatively, a sample for measuring mRNA corresponding to the aforementioned gene can be obtained by extracting mRNA from this lysate. The use of a commercially available kit is convenient for extracting mRNA or preparing a lysate of eosinophils.

As an embodiment of the present method of testing for allergic diseases, the expression level of the gene that serves as an index may be measured, without isolation of eosinophils, in whole blood or peripheral blood leukocyte population. In this case, by correcting the measured values, the change of gene expression levels in cells can be determined. For example, based on the measured value of the expression level of a gene (housekeeping gene) that is specifically expressed in eosinophils and whose expression level is not widely altered regardless of the cellular conditions, the measured value of the expression level of the gene serving as an index in this invention can be corrected.

Alternatively, in the case where the protein to be detected is a secretory protein, comparison of the expression level of a gene encoding the protein is accomplished by measuring the amount of the target protein contained in body fluid sample, such as blood and serum, in a subject.

When the result of the test for an allergic disease of this invention shows an elevated expression level of the gene of this invention especially in a patient with an allergic disease such as atopic dermatitis, allergic symptoms are presumed to be improving together with the decrease of eosinophils.

Furthermore, this invention relates to an allergic disease animal model, wherein said animal is a transgenic non-human animal showing decreased expression level of the polynucleotide of (a) or (b) in eosinophil cells:

(a) a polynucleotide encoding an NOR-1 receptor protein; and

(b) a polynucleotide encoding a protein whose expression in eosinophils increases in association with the decrease of eosinophils in the remission stage of atopic dermatitis, wherein said polynucleotide hybridizes under stringent conditions with a polynucleotide encoding an NOR-1 receptor protein.

According to this invention, a decrease in expression level includes a knockout condition in which the function of the gene has been substantially diminished. Herein, the condition in which the function of the gene has virtually suppressed refers to a condition in which neither expression of the gene nor the activity of the protein encoded by this gene is observed. The expression level of the gene can be confirmed by quantitative PCR such as those shown in Examples. Furthermore, that substantially no activity of the translation product protein is detected can be confirmed by comparison to a normal condition.

Such a transgenic animal includes animals that are incapable of expressing the intact activity of the protein, for example, due to artificial mutation of the amino acid sequence and introduction of a stop codon by introducing a mutation into the coding region of the gene. Examples of mutation in the amino acid sequence are substitution, deletion, insertion and addition of amino acid(s). In addition, by mutagenizing the transcriptional regulatory region of the gene, the expression itself of the gene of this invention can be controlled.

Methods for obtaining transgenic animals with a particular gene as a target are known. That is, a transgenic animal can be obtained by a method where the gene and ovum are mixed and treated with calcium phosphate; a method where the gene is directly introduced into the nucleus of oocyte in the pronuclei using a micropipette under a phase contrast microscope (microinjection method; US Patent No. 4873191); a method where embryonic stem cells (ES cells) are used; etc. Furthermore, a method has been developed for infecting ovum with a gene-inserted retrovirus vector, a sperm vector method for transducing a gene into ovum via sperm, etc. The sperm vector method is a gene recombination technique for introducing an exogenous gene by fertilizing the ovum with sperm after the exogenous gene has been incorporated into the sperm by adhesion, electroporation, and so on (M. Lavitranoet, et al. Cell, 57, 717, 1989).

Transgenic animals of the present invention can be produced using all the vertebrates except for humans. More specifically, transgenic animals having various transgene and modified gene

expression levels are produced using vertebrates such as mice, rats, rabbits, miniature pigs, goats, sheep or cattle.

Examples of transgenic animals of this invention include a knockout animal in which expression of a homologue of the human NOR-1 gene comprising the nucleotide sequence of SEQ ID NO: 1 is inhibited. Observation of the phenotype of the knockout animal specifically tells the function of the gene that was knocked out. The NOR-1 gene comprising the nucleotide sequence of SEQ ID NO: 1 showed increased expression in eosinophils in the remission stage of atopic dermatitis associated with the decrease of eosinophils in humans. Therefore, the animal in which a homologue of this gene is knocked out is useful as an animal model for allergic diseases.

For example, if the knockout animal of this invention develops dermatitis, or indicates change in measured values relating to some sort of allergic diseases, a screening system to search for a compound having the function to allow recovery therefrom can be constructed.

A method of producing a knockout animal is well known. For example, the method of producing a knockout mouse by performing homologous recombination using embryonic stem cells, and selecting the embryonic stem cells in which one of the alleles is modified or destroyed, is known. More specifically, a chimeric animal containing cells derived from an embryonic stem cell and cells derived from an embryo, is obtained, for example, by inserting a genetically manipulated embryonic stem cell into a fertilized egg. When this chimeric animal (chimera refers to a single individual formed from somatic cells derived from two or more fertilized eggs) is crossed with a normal mouse, a heterozygote in which one of the alleles is modified or destroyed in its entirety can be produced. Furthermore, a homozygote can be produced by crossing heterozygotes. The transgenic animals of this invention include both the heterozygote and the homozygote.

Homologous recombination refers to a mechanism of genetic recombination that occurs between two genes having the same or very similar nucleotide sequences. PCR can be used to select cells that have undergone homologous recombination. PCR using a portion of an insert gene and a portion of the region in which insertion is expected

as primers, can confirm the occurrence of homologous recombination in cells that produce amplification products. Furthermore, when inducing homologous recombination of a gene expressed in an embryonic stem cell, neomycin resistance gene is linked to a transgene and the gene is introduced into a cell to make the cell neomycin resistant, to thereby easily select the cells. This and other known methods and modified methods thereof can be used for selection of cells.

As shown in the Examples described below, the present inventors succeeded in establishing a transgenic (TG) mouse in which the human NOR-1 gene expression is actually induced by an actin promoter. The TG mouse mentioned above is an example of a preferred embodiment of the transgenic animal of this invention.

In addition to the screening of pharmaceutical agents for the treatment or prevention of allergic diseases described below, the transgenic animals of this invention are useful for elucidating the mechanism of allergic diseases and for testing the safety of screened compounds.

According to the present invention, the expression levels of the NOR-1 gene were revealed to rise in the eosinophils of atopic dermatitis patients in the remission stage associated with the decrease of eosinophils. Therefore, animals in which the expression level of the NOR-1 gene or genes functionally equivalent thereto in eosinophil cells has been artificially lowered can be used as allergic disease model animals. The decrease of expression level in eosinophils includes decrease in the expression level of the genes in the entire population of leukocytes. In other words, this phrase includes the decreased expression level of the aforementioned genes not only in eosinophils but also in the entire population of leukocytes. In the present invention, a functionally equivalent gene normally refers to a gene of either (a) or (b) described above. More specifically, examples of functionally equivalent genes of this invention include genes that hybridize under stringent conditions to a gene that encodes NOR-1. Generally, the following conditions can be indicated as the stringent conditions of this invention. Stringent conditions generally mean hybridization in 4x SSC at 65°C followed by washing with 0.1x SSC at 65°C for 1 hour. Temperature

conditions for hybridization and washing that greatly influence stringency can be adjusted by the melting temperature (Tm). Tm varies with the ratio of constitutive nucleotides in the hybridizing base pairs and the composition of hybridization solution (concentrations

5 of salts, formamide and sodium dodecyl sulfate). Therefore, considering these conditions, those skilled in the art can select an appropriate condition to achieve an equal stringency from their experience or through experimentation.

For example, the aforementioned transgenic animals may be used

10 as the animal model of this invention.

Furthermore, the present invention provides a method for detecting the influence of a candidate compound on the expression level of the polynucleotide of this invention. According to this invention, the expression level of the NOR-1 gene is significantly

15 increased in eosinophils of atopic dermatitis patients in the remission stage associated with the decrease of eosinophils. Therefore, based on the method for detecting the influence on the expression level of the gene, therapeutic drugs for allergic diseases can be obtained by selecting compounds that increase its expression

20 level. Herein, compounds that increase the expression level of a gene refer to compounds that have the function of inducing any one of the steps selected from gene transcription, translation and expression of protein activity. The present invention further provides a method for detecting the activity of the NOR-1 gene product protein

25 (transcriptional activation ability) in addition to the expression level of the NOR-1 gene. By selecting compounds that increase the activity of the NOR-1 gene product protein (transcriptional activation ability), therapeutic drugs for allergies can be devised.

The method for detecting the influence of a candidate compound

30 on the expression level of the polynucleotide according to this invention can be performed *in vivo* or *in vitro*. In order to detect an *in vivo* influence, an appropriate test animal is used. For example, an animal model for an allergic disease or an animal model for an allergic disease that is a transgenic non-human animal in which the

35 expression of the aforementioned gene (a) or (b) is inhibited in eosinophils can be used as the test animal. Detection of *in vivo*

influence on the expression level based on the present invention can be performed, according to the following steps of:

(1) administering the candidate compound to a test animal; and

(2) measuring, in the eosinophil cells of the test animal, the expression level of a polynucleotide of (a) or (b) described above:

For example, transgenic animals in which the expression of the NOR-1 gene has been decreased by expressing an antisense of the NOR-1 gene can be used as a test animal in the method of detection of this invention. Such transgenic animals may be produced as follows: first, an antisense RNA expression vector is constructed by inserting the full-length or partial sequence of the NOR-1 gene downstream of an appropriate promoter sequence in reverse direction. This expression vector is introduced into the nucleus to express the antisense of the NOR-1 gene. Thus, a transgenic animal with decrease NOR-1 gene expression can be obtained. When the expression vector contains a promoter whose transcription may be regulated by an appropriate substance (agent), the expression level of the NOR-1 gene in a transgenic animal can be controlled by administering that substance.

The influence of a candidate compound for a pharmaceutical agent on the expression level of the NOR-1 gene can be detected by administering the candidate compound for a pharmaceutical agent to an animal model in which the expression of the NOR-1 gene is decreased as described above and by monitoring the effect of the compound on the expression of the NOR-1 gene in the eosinophils of the animal model.

The method of screening of this invention allows selection of pharmaceutical agents involved in various ways in the expression of the NOR-1 gene. Specifically, for example, candidate compounds for pharmaceutical agents having any of the following function can be discovered:

activation of a signal transduction pathway that causes expression of the NOR-1 gene;

increase of transcription activity of the NOR-1 gene;

inhibition of degradation or stabilization of the transcription product of the NOR-1 gene; etc.

An *in vitro* detection can be performed, by a method where a

candidate compound is contacted with cells expressing a gene of above-described (a) or (b) to detect the expression level of the gene. More specifically, the method may be carried out according to the following steps of:

5   (1) contacting a candidate compound with cells that express the polynucleotide of the above-described (a) or (b); and
(2) measuring the expression level of the polynucleotide of the above-described (a) or (b).

In this invention, cells to be used in the step (1) can be
10   obtained by inserting these polynucleotides into an appropriate expression vector and then transfecting suitable host cells with the vector. Any vectors and host cells may be used as long as they are capable of expressing the gene of this invention. Examples of host cells in the host-vector system are *Escherichia coli*, yeast cells,
15   insect cells, animal cells, and available vectors usable for each can be selected.

Vectors may be transfected into the host by biological, physical or chemical methods. Examples of the biological methods include methods using virus vectors; methods using specific receptors; and
20   the cell-fusion method (HVJ (hemagglutinating virus of Japan; Sendai virus) method, the polyethylene glycol (PEG) method, the electric cell fusion method and microcell fusion method (chromosome transfer)). Examples of the physical methods include microinjection, electroporation and using a gene particle gun. The chemical methods
25   are exemplified by the calcium phosphate precipitation method, the liposome method, the DEAE-dextran method, the protoplast method, the erythrocyte ghost method, the erythrocyte membrane ghost method and the microcapsule method.

In the detection method of this invention, leukocyte cell lines
30   can be used as cells for expressing the polynucleotide of the aforementioned (a) or (b). Examples of leukocyte cell lines are cell lines derived from leukocytes, such as Eol, YY-1, HL-60, TF-1 and AML14.3D10. Among the leukocyte cell lines, cell lines derived from eosinophils are preferred for the detection method of this invention.
35   Examples of cell lines derived from eosinophils include Eol, YY-1 and AML14.3D10.

Eol (Eol-1: Saito H et al., Establishment and characterization of a new human eosinophilic leukemia cell line. Blood 66, 1233-1240, 1985) can be obtained from Hayashibara Research Institute. Similarly, YY-1 (Ogata N et al., The activation of the JAK2/STAT5 pathway is commonly involved in signaling through the human IL-5 receptor. Int. Arch. Allergy Immunol., Suppl 1, 24-27, 1997) is available from The Institute of Cytosignal Research. Furthermore, AML14.3D10 (Baumann MA et al., The AML14 and AML14.3D10 cell lines: a long-overdue model for the study of eosinophils and more. Stem Cells, 16, 16-24, 1998) is commercially available from Paul CC at Research Service, VA Medical Center, Dayton, Ohio, USA.

In addition, by culturing for about 1 week in the presence of butyric acid, HL-60 clone 15 (ATCC CRL-1964), which is an undifferentiated leukocyte cell line, can differentiate into eosinophils to give an eosinophil cell line. Eosinophils can be detected due to their morphological characteristic of being polymorphonuclear and having eosinophilic granules. Morphological observations are performed by Giemsa staining and Difquick staining. Generally, human leukocyte cell line containing eosinophils can be established by cloning immortalized cell sample from a leukemia patient. Therefore, those skilled in the art can obtain eosinophil cell lines by a conventional method when necessary. The method of screening involves addition of a candidate compound with the aforementioned leukocyte cell line. Then, the expression levels of the polynucleotides of (a) or (b) in the leukocyte cell line are measured and a compound that increases the expression level of the gene is selected.

Transformed cells in which the expression of the polynucleotide of aforementioned (a) or (b) is modified can be used as cells for the *in vitro* detection method. Examples of such transformed cells are cells transformed with an expression vector for an antisense of the polynucleotide. The cell transformed with an antisense expression vector can be obtained according to a principle similar to that for the production of the aforementioned transgenic animal. Using the obtained transformed cell, the influence of the candidate compound on the expression level of the gene can be detected.

In the method of the present invention, the expression levels of polynucleotides of described above (a) or (b) can be compared by detecting the expression levels of not only proteins encoded by these genes but also by the corresponding mRNAs. For the comparison of the expression level using mRNA, the step of preparing mRNA sample as described above is conducted in place of the step of preparing a protein sample. Detection of mRNA and protein can be carried out according to the known methods as described above.

Furthermore, it is possible to obtain the transcriptional regulatory region of the NOR-1 gene and construct a reporter assay system. Reporter assay system means a system of screening for a transcriptional regulatory factor that acts on the transcriptional regulatory region using the expression level of a reporter gene that is located downstream of the transcriptional regulatory region and expressed under the control of the regulatory region as an index.

A transcriptional regulatory region is exemplified by promoter, enhancer, as well as CAAT box and TATA box, which are usually found in the promoter region. Examples of the reporter gene include the chloramphenicol acetyltransferase (CAT) gene, the luciferase gene and growth hormone genes.

A transcriptional regulatory region of the NOR-1 gene can be obtained using conventional methods as follows. Specifically, first, based on the nucleotide sequence described in SEQ ID NO: 1, a human genomic DNA library, such as BAC library and YAC library, is screened by a method using PCR or hybridization to obtain a genomic DNA clone containing the sequence of the cDNA. Based on the sequence of the resulting genomic DNA, the transcriptional regulatory region of the NOR-1 gene is predicted and obtained. The obtained transcriptional regulatory region is cloned to be localized upstream of a reporter gene to prepare a reporter construct. The resulting reporter construct is introduced into a cultured cell strain to prepare a transformant for screening. By contacting a candidate compound with this transformant to detect the expression of a reporter gene, it is possible to assess the effect of the candidate compound on the transcriptional regulatory region.

Based on the method of detecting the influence on the expression

level of the polynucleotides of this invention, it is possible to carry out screening for a compound that alters the expression level of the polynucleotides. This invention relates to a method of screening for a compound that alters the expression level of a

5 polynucleotide of above-described (a) or (b), comprising of the following steps.

The present invention provides a method of screening for a compound that increases the expression level of a polynucleotide of above-described (a) or (b), the method comprising the steps of

10 detecting the influence of a candidate compound on the expression level of the polynucleotide *in vivo* and/or *in vitro,* and selecting a compound that increases the expression level compared to a control.

Alternatively, this invention relates to a method of screening for a compound that acts on the transcriptional regulatory region

15 by the reporter assay utilizing the transcriptional regulatory region of the NOR-1 gene. Based on the results of reporter assay according to this invention, by selecting a compound that increases the expression level of the reporter gene compared to a control, it is possible to obtain a compound that induces the expression of the NOR-1

20 gene. Furthermore, the present invention relates to a method of screening for agonists or antagonists that bind to the ligand-binding domain.

The NOR-1 (MINOR) receptor protein discovered by the present inventors as a protein associated with allergic diseases is an orphan

25 receptor and hitherto, none of its native ligand activator has been found. The ligand activator of the NOR-1 protein is considered to directly activate NOR-1 in eosinophils, and promote apoptosis. Therefore, a ligand activator of the NOR-1 receptor is expected to serve as a therapeutic agent for allergic diseases. Generally, a

30 ligand for the receptor can be obtained by searching compounds that bind to the receptor protein.

The present invention provides a method of screening for candidate compounds for therapeutic agents for an allergic disease, which comprises selecting compounds that may bind to the NOR-1 protein.

35 In this method, the NOR-1 receptor protein is contacted with a test compound, then the binding activity between the NOR-1 receptor protein

and the test compound is measured, and the compound that binds to the NOR-1 receptor protein is selected. Furthermore, agonists and antagonists can be selected by measuring the binding and by the transcription activity of NOR-1.

5   The NOR-1 receptor protein of this method includes its partial peptides. The measurement of the binding activity between the NOR-1 receptor protein and a test compound in the method described above can be carried out by using methods known to those skilled in the art.

10   For example, when the compound that binds to NOR-1 is a protein, West-Western blotting can be performed as the screening method of this invention. Specifically, a cDNA library that uses a phage vector (λgt11, ZAPII, etc.) is constructed from tissues or cells predicted to express a protein (test protein) that binds to the NOR-1 protein,

15   this library is expressed on LB-agarose and expressed proteins are immobilized onto a filter. Then, the NOR-1 protein is purified as a biotin labeled protein or as a fusion protein with the GST protein, and reacted with the abovementioned filter. The binding activity can be evaluated due to the detection of plaques expressing the test

20   protein using streptavidin, anti-GST antibodies.

Furthermore, another embodiment of the method of screening for a candidate compound for a therapeutic agent for an allergic disease of this invention includes the following steps of:

25   (1) providing cells transfected with (a) a DNA that can express a fusion protein of an NOR-1 receptor protein or its ligand binding domain and a transcription regulatory region binding protein, and (b) a DNA having a structure such that a reporter gene is operably linked downstream of a DNA sequence to which the transcription

30   regulatory region binding protein binds;
(2) contacting the cell with a test compound;
(3) measuring the activity of the reporter gene; and
(4) selecting a compound that changes the activity.

The phrase "operably linked" in the above-mentioned method

35   refers to a condition in which the reporter gene is bound such that it may be expressed when the NOR-1 receptor protein or the ligand

binding domain of the protein binds to a ligand of the receptor protein or to a ligand-like compound. Generally, GAL4 protein can be preferably used as the "transcription regulatory region binding protein" in the above-mentioned method. Furthermore, GAL4-binding

5   DNA region can be mentioned as an example of the "DNA sequence to which a transcription regulatory region binding protein binds". Additionally, the screening method of the present invention can be performed by a high throughput method.

In a preferred embodiment of the screening method, screening

10   may be performed using the "two-hybrid system" (for example, "MATCHMAKER Two-Hybrid System", "Mammalian MATCHMAKER Two-Hybrid Assay Kit", "MATCHMAKER One-Hybrid System" (all of which are manufactured by Clontech), "HybriZAP Two-Hybrid Vector System" (Stratagene), and methods reported in the literature (Dalton S, and

15   Treisman R (1992) "Characterization of SAP-1, a protein recruited by serum response factor to the c-fos serum response element." Cell 68, 597-612). More specifically, the method of the present invention may be performed as described below, but it is not to be construed as being limited thereto, and those skilled in the art can

20   appropriately modify the method illustrated below to achieve this invention.

In the two-hybrid system, the NOR-1 protein or its partial peptide is normally fused with the GAL4 DNA binding domain and expressed in yeast cells. Using cells that are predicted to express

25   a protein that binds to the NOR-1 protein or to its partial peptide, a cDNA library is constructed which expresses the protein as a fusion protein fused with VP16 or GAL4 transcriptional activating region. Then, this library is introduced into yeast cells. Finally, cDNAs that stem from the library are isolated from detected positive clones

30   (when a protein that binds to the NOR-1 protein or partial peptides including its ligand binding domain is expressed in yeast cells, a reporter gene is activated due to the binding of the protein and the NOR-1 protein or the partial peptide, and thus a positive clone can be detected). Proteins encoded by the cDNAs can be obtained by

35   expressing the isolated cDNAs following transfection into *E. coli*. Thus, proteins that bind to the NOR-1 protein or its partial peptide

and genes encoding the proteins may be prepared. Examples of the reporter genes that can be used in the two-hybrid system include, in addition to the HIS3 gene, the Ade2 gene, the LacZ gene, the CAT gene, the luciferase gene and the Plasminogen activator inhibitor type 1 (PAI-1) gene. However, they are not limited to these examples. Apart from yeast cells, the screening using the two-hybrid method can also be performed using mammalian cells or such.

The present inventors constructed a high throughput system that can screen ligands that increases the transcriptional activating function of the NOR-1 protein by applying a two-hybrid system that uses mammalian cells. This system is an improvement over the conventional mammalian two-hybrid system, and the outline of this system is shown in Fig. 3 (for details, see Examples below).

Preferably, the screening method of this invention can be performed using the aforementioned high throughput system developed by the present inventors. The present inventors suggested that NOR-1 differs from the other subfamilies (α and β) in that, not only the ligand-binding domain, but also the AF1 region in the vicinity of the N-terminal (Fig. 4) is important for its transcriptional activity. Therefore, in the above-mentioned method, the NOR-1 protein used for forming a fusion protein with GAL4 is preferably a full-length protein and not a protein comprising only the ligand-binding domain.

NOR-1 expression is induced under conditions where leukocytes are hyperactive, as in atopic dermatitis peripheral blood. It is very likely that apoptosis is induced in the cells because of such induction of NOR-1 expression. _In vivo_ ligands may exist in locations where the nuclear receptor is highly expressed. Therefore, the present inventors performed screening according to the above-mentioned method using small molecule lipid-soluble mediators predicted to be produced under such condition as ligand candidate test compounds. Accordingly, the present inventors succeeded in obtaining prostaglandin A_2, prostaglandin A_1, 16,16-dimethyl prostaglandin A_2, 15(R)-15-methyl prostaglandin A_2, 16-phenoxy tetranor prostaglandin A_2, 17-phenyl trinor prostaglandin A_2, 15-deoxy-delta 12,14-prostaglandin J_2, 8-iso prostaglandin A_1 and such as ligand activators amongst the lipid-soluble mediators. These compounds are prostaglandins having

a cyclopentenone structure. This shows that ligand activators that up regulate the transcriptional activating function of NOR-1(MINOR) can be obtained via the method of this invention.

Screening of compounds that bind to the NOR-1 protein can also
5   be performed using affinity chromatography. For example, the NOR-1 protein is immobilized on an affinity column carrier, and a test sample predicted to express a protein that binds to the NOR-1 protein is applied thereto. Test samples that can be used in this case include, cell extract and cell lysate. After applying the test sample, the
10   column is washed to prepare a protein that was bound to the NOR-1 protein.

Regarding the obtained protein, a DNA encoding the protein can be obtained by analyzing the amino acid sequence of the protein, synthesizing oligo DNAs based on the analyzed sequence, and then
15   screening a cDNA library using the DNA as a probe.

In the present invention, as a means for detecting or measuring bound compound, a biosensor utilizing surface plasmon resonance phenomenon may be used. A biosensor (for example, BIAcore, Pharmacia) utilizing the surface plasmon resonance phenomenon allows
20   real-time observation of the interaction between the NOR-1 protein and a test compound as a surface plasmon resonance signal. Therefore, using biosensors such as BIAcore, the binding between the NOR-1 protein and the test compound can be evaluated.

Isolation of compounds that bind to the NOR-1 protein may be
25   routinely carried out by those skilled in the art. Methods other than those mentioned above include a method for screening molecules that bind to the protein of this invention by acting, on immobilized NOR-1 protein, synthetic compounds, natural products bank or a random phage peptide display library.

30   A cell used for the method of detecting the influence of a candidate compound on the expression level and transcriptional activation mechanism of the NOR-1 gene according to the present invention, and a polynucleotide or antibody for examining the expression level of the gene can be combined as a detection kit for
35   this method. Candidate compound(s) used as positive or negative control and instructions may be included in the kit. A kit for

detecting the influence of a candidate compound on the expression level and transcriptional activation mechanism of the NOR-1 gene based on the present invention may be utilized as a kit for screening compounds that modify the expression level or transcriptional

5 activation mechanism of the NOR-1 gene.

Test candidate compounds that can be used in the screening method of this invention include, without limitation, compound preparations synthesized by chemical methods, such as steroid derivatives; compound preparations synthesized by combinatorial

10 chemistry; mixtures containing multiple compounds such as extracts from animal or plant tissues, or microbial cultures; purified proteins; expression products of gene libraries; and libraries of synthetic peptides. Furthermore, in the method of screening for compounds that bind to the NOR-1 protein of the present invention,

15 without limitation, it is preferable to use small molecule lipid-soluble mediators as the test candidate compounds.

Compounds selected by the screening method of this invention are useful as therapeutic agents for an allergic disease. The expression of the NOR-1 gene increases in eosinophils with the

20 decrease of eosinophils in the remission stage of atopic dermatitis. Therefore, compounds that can increase the expression or function of this gene are expected to have a function to suppress symptoms of atopic dermatitis. Furthermore, compounds selected by the screening method of this invention are expected to serve as

25 therapeutic agents for allergic diseases that have a completely novel functional mechanism involving NOR-1 activation accompanied with eosinophil apoptosis induction. Therefore, the present invention provides a therapeutic agent for an allergic disease containing, as an active ingredient, a compound that can be obtained by the screening

30 method of this invention. The above-mentioned compound includes compounds in which a portion of the structure of the compound that may be isolated using the screening method of this invention is altered by addition, deletion and/or replacement. As described above, among lipid-soluble mediators, prostaglandins having a cyclopentenone

35 structure were found by the present inventors as compounds that enhance the transcriptional activation ability of NOR-1 (ligand

activator of NOR-1). Therefore, examples of therapeutic agents for allergic diseases of this invention preferably include, therapeutic agents for allergic diseases containing, as an active ingredient, a prostaglandin having a cyclopentenone structure that is obtainable by the screening method of this invention. Specific examples of the prostaglandins include, prostaglandin A_2, prostaglandin A_1, 16,16-dimethyl prostaglandin A_2, 15(R)-15-methyl prostaglandin A_2, 16-phenoxy tetranor prostaglandin A_2, 17-phenyl trinor prostaglandin A_2, 15-deoxy-delta 12,14-prostaglandin J_2 and 8-iso prostaglandin A_1.

Furthermore, substances having ligand activity of the NOR-1 receptor of the present invention appear to induce apoptosis of eosinophils and may have antiallergic effects. Therefore, the present invention provides apoptosis inducing agents containing a ligand of the NOR-1 receptor as an active ingredient, as well as therapeutic agents for allergic diseases containing a ligand of the NOR-1 receptor as an active ingredient. The apoptosis inducing agent of the present invention is preferably an apoptosis inducing agent for eosinophils.

Examples of ligands for the NOR-1 receptor include the above-mentioned prostaglandins having a cyclopentenone structure, and compounds listed in Tables 14 to 58 shown below. In addition, compounds that influence the transcription of NOR-1 include, for example, compounds indicated in the literature (Abayratna Wansa KS, Harris JM, Yan G, Ordentlich P, Muscat GE, "The AF-1 domain of NOR-1/NR4A3 mediates trans-activation, coactivator recruitment and activation by the purine anti-metabolite 6-Mercaptopurine"; J Biol Chem. 2003 Apr 22 [E-publication ahead of print]).

Moreover, synthetic ligands of NOR-1(MINOR) can be easily inferred by those skilled in the art from docking studies with the three-dimensional structure of NOR-1, and then are synthesized and developed.

Generally, the term "docking study" refers to searching for compounds and conformations that fit into a ligand-binding domain from a 3-dimensional database comprising several hundred thousand compounds using 3D query pharmacophore model based on the three-dimensional structure of a receptor. The docking study is

performed, for example, according to procedures (1) to (4) described below:

(1) constructing a 3D structure of a protein by Modeler (homology model);

(2) searching a binding site by C2. LigandFit;

(3) constructing a pharmacophore query for the binding site by C2· SBF; and

(4) searching in 3D database using the pharmacophore query.

Literature relating to 3D Pharmacophore search includes, Pharmacophore Perception, Development, and Use in Drug Design (Iul Biotechnology Series, 2)-US-ISBN:0963681761 (Hardcover) Guner, Osman F. (Ed.)/Publisher: Intl. Univ. Line Published 1999/12.

Pharmaceutical agents containing such synthetic ligands as an active ingredient are also included in the therapeutic agents for allergic diseases of this invention. Furthermore, by subjecting a synthetic ligand described above to the method of this invention as a test candidate compound, one can evaluate whether the synthetic ligand is a true ligand or not.

Furthermore, based on the first finding that the expression of the NOR-1 receptor of this invention is specifically induced in eosinophils, the present inventors searched for small molecule ligands of the receptor. More specifically, the binding site of the PGA derivative to the NOR-1 ligand-binding region was simulated using the pharmacophore model, and based on the structure activity relationship information on the reporter system of the PGA derivative, compounds other than the PGA derivatives that match the binding pocket were selected from the database. Thus, compounds selected as described above are included as ligands of the NOR-1 receptor of this invention. More specifically, the ligands include compounds shown in Tables 14 to 58. These compounds may be more useful than agonist antibodies against the receptor of this invention.

The present inventors further discovered that eosinophil CD30 ligand stimulation increases the expression of the NOR-1 gene. Thus, the present invention provides an expression-inducing agent for the NOR-1 gene, which includes a ligand of the eosinophil CD30 receptor. The expression inducing agent is expected to serve as a therapeutic

agent for an allergic disease that functions by inducing apoptosis of eosinophils via the regulation of the expression of downstream genes of Nor1 in eosinophils.

The therapeutic agents for an allergic disease of this invention can be formulated by mixing an active ingredient with a physiologically acceptable carrier, excipient, diluent or such. The therapeutic agent for an allergic disease of this invention can be administered orally or parenterally with the purpose of improving allergic symptoms.

Oral drugs can take any dosage forms selected from granules, powder, tablets, capsules, solution, emulsion, suspension and so on. Examples of parenteral agents include injections, suppositories and ointments. Injections may include subcutaneous injection, intramuscular injection and intraperitoneal injection.

The dosage of the therapeutic agent for an allergic disease according to the invention may vary depending upon the age, sex, body weight and symptoms of a patient; treatment effects; method for administration; treatment duration; type of active ingredient contained in the pharmaceutical composition etc. Generally, it can be administered in the range of 0.1 mg to 500 mg, preferably 0.5 mg to 20 mg per dose for an adult. However, since the dose varies according to the specific condition, an amount less than the above-described dosage may be sufficient in certain cases and a dosage exceeding the above-described range may be required in others.

Furthermore, the present inventors discovered that apoptosis of cells is induced due to the rise in the expression of the NOR-1 receptor protein. Therefore, apoptosis can be induced by activating the NOR-1 protein in cells. Thus, the present invention provides a method of inducing apoptosis of cells, which comprises activation of the NOR-1 receptor protein in these cells. The method mentioned above also includes a method wherein the induction of apoptosis of cells is conducted through the activation of the expression of the NOR-1 gene.

In a preferred embodiment of the present method, apoptosis is induced by contacting cells with a compound or a prostaglandin having a cyclopentenone structure that can be obtained by the screening

method of this invention. The cells in the method of this invention are preferably eosinophils. The number of peripheral blood eosinophils is known to decrease in the remission stage during the treatment of atopic dermatitis. Therefore, an allergic disease may be treated by specifically leading eosinophils to cell death utilizing the method of the present invention. Thus, the present method is useful in treating an allergic disease.

Furthermore, since compounds or prostaglandins having a cyclopentenone structure that can be obtained by the screening method of this invention are considered to have a function to induce apoptosis, the present invention also provides apoptosis inducing agents that include these compounds.

The present invention provides a gene whose expression differs in eosinophils between the exacerbation stage and the remission stage of atopic dermatitis patients. The use of the expression of the gene of this invention as an index enables testing for an allergic disease and screening for a candidate compound for a therapeutic agent.

The expression level of the allergic disease-associated gene of the present invention can be conveniently determined, regardless of the types of allergens. Therefore, comprehensive pathological conditions of allergic reactions can be understood.

In addition, according to the method of testing for allergic diseases of the present invention, the expression level of the gene can be analyzed using peripheral blood eosinophils as a specimen. Therefore, the method of testing for allergic diseases of the present invention is less invasive towards patients. Year after year, high throughput methods and cost effective methods are being developed in gene analysis technology. Therefore, it is expected that in the near future the method of testing for allergic diseases of the present invention will become an important bedside diagnostic tool. Accordingly, the method of the present invention is highly valuable in diagnosis.

Furthermore, the screening method of the present invention is carried out using, as an index, a genetic function closely associated with the increase and decrease of eosinophils that is a representative diagnostic marker for atopic dermatitis. Therefore, compounds that

can be found by this screening method are expected to be useful for pathological regulation of a wide variety of allergies.

Moreover, the therapeutic agents for an allergic disease provided by the present invention are useful as pharmaceutical agents having a completely novel functional mechanism, which involves activation of NOR-1 accompanied with the induction of apoptosis of eosinophils.

Hereinafter the present invention is specifically illustrated below with reference to Examples, but is not to be construed as being limited thereto.

EXAMPLE 1

Differential display analysis

Screening was performed in order to find novel therapy related genes or genes useful for diagnosis, whose expression in hemocytes isolated from the peripheral blood of an atopic dermatitis patient in the exacerbation stage differs from that of remission due to drug therapy, etc.

(1) Test subject

Table 1 shows the profiles of seven atopic dermatitis patients whose blood samples were drawn. Allergen non-specific (Total IgE), mite-specific and cedar-specific IgEs were measured by the EIA method. More specifically, the test sera were allowed to react to anti-human IgE antibody-bound cap to bind thereto allergen non-specific IgE antibody or mite- or cedar-specific IgE antibodies in the sera. Next, β-D-galactosidase-labeled anti-human IgE antibody and a substrate solution (4-methylumbelliferyl-β-D-galactopyranoside) were added and allowed to react to produce a fluorescent substance. The reaction was quenched by adding a quenching solution, and the antibody concentration was determined from the fluorescence intensity of a simultaneously measured standard IgE. L-lactate dehydrogenase (LDH) was measured by the UV method (Wroblewski-La Due method) based on the rate of decrease of NADH caused by the reaction of pyruvic acid with NADH is calculated from decrease in absorbance. L-type Wako LDH (Wako Pure Chemicals) and 7170-type automatic analyzer (Hitachi) were

used for measuring the LDH values. The number of eosinophils was measured by microscopic examination and automatic hemocyte analyzer SE-9000 (RF/DC impedance system, Sysmex) using 2 ml of EDTA-added blood as the sample.

5

Table 2

Patient number	1		2		3		4		5		6		7	
Donor ID	PA00002		PA00068		PA00069		PA00070		PA00071		PA00073		PA00164	
Condition	Exacerbation stage	Remission stage	Exacerbation stage	Remission stage	Exacerbation stage	Remission stage	Exacerbation stage	Remission stage	Exacerbation stage	Remission stage	Exacerbation stage	Remission stage	Exacerbation stage	Remission stage
T-IgE	6100	7100	2600	2100	13000	20000	15000	15000	9300	9200	17000	8800	2100	1600
Mite	82.1	73.8	66.4	>100	72.2	66.7	85.9	90.9	74.6	70.8	88	>100	>100	82.8
Cedar	57.1	77.2	14.4	19.7	15.2	22.5	61.9	59.6	64.2	71.1	18.3	9.27	6.51	3.61
LDH	910	475	293	296	398	250	173	182	534	297	620	598	343	393
Eosinophil (%)	16	11.7	23.2	10.1	16	6.2	8.6	12.1	28.2	13.4	13.4	12.3	12.9	10.6
Eosinophil (/mm³)	1620	611	1420	468	2070	527	738	752	1830	972	945	846	898	847
Internal use	ALDECIN Theodur INTAL inhalant,	inhalant, ZADITEN	ZADITEN				ZADITEN, Shofusan	ATARAX P	CELBECT	INTAL oral preparation	ZADITEN DS	INTAL →none	PREDONINE (only 181)	Theolong, ALDECIN
External use	Body: Zalucs Face: LOCOID		Body: LOCOID Face: nonsteroid		Body: Zalucs Face: nonsteroid		Body: LOCOID Face: nonsteroid		Body: LOCOID Face: nonsteroid		Body: Zalucs →RINDERON V Face: LOCOID		Body: Zalucs Face: LOCOID	
Other diseases	Asthma (moderate)								Asthma (moderate)				Asthma (severe/mild)	

(2) Differential display analysis

A 3% dextran solution was added to whole blood drawn from a patient, and this was left to stand at room temperature for 30 minutes to precipitate erythrocytes. The upper layer leukocyte fraction was collected, layered on top of Ficoll solution (Ficoll-Paque PLUS; Amersham Pharmacia Biotech), and centrifuged at 1500 rpm for 30 minutes at room temperature. The granulocyte fraction that collected in the lower layer was reacted with CD16 antibody magnetic beads at 4°C for 30 minutes, and cells that had eluted without being trapped in the separation using Magnetic cell sorting (MACS) were used in the experiment as eosinophils.

Eosinophils prepared as described above were added to Isogen (Nippon Gene; Wako Pure Chemicals). From this resulting solution, RNA was separated according to the protocol attached to Isogen. Chloroform was added, the mixture was stirred and centrifuged, and the aqueous layer was collected. Next, isopropanol was added, the mixture was stirred and centrifuged and the precipitated total RNA was collected. DNase (Nippon Gene; Wako Pure Chemicals) was added to the collected total RNA, the mixture was reacted at 37°C for 15 minutes, and RNA was collected by phenol-chloroform extraction followed by ethanol precipitation.

Fluorescent Differential Display (abbreviated to DD) analysis using the total RNA thus prepared was carried out according to the literature (T. Ito et al., 1994, FEBS Lett. 351: 231-236). The total RNA was reverse transcribed to obtain cDNA. In the first DD-PCR, 0.2 µg each of the total RNA was used for three types of anchor primers to synthesize cDNAs. In the second DD-PCR, 0.4 µg each of the total RNA was used for the synthesis of cDNAs using three types of anchor primers. In both cases, the cDNAs were diluted to a final concentration equivalent to 0.4 ng/µl RNA and used for further experiments. The DD-PCR was carried out using an amount of cDNA equivalent to 1 ng RNA per reaction. The reaction mixture composition is shown in Table 3.

Table 3

cDNA (equivalent to 0.4 ng/µl RNA)	2.5 µl
Arbitrary primer (2 µM)	2.5 µl
10x AmpliTaq PCR buffer	1.0 µl
2.5 mM dNTP	0.8 µl
50 µM anchor primer (GT15A, GT15C, or GT15G)	0.1 µl
Gene Taq (5 U/µl)	0.05 µl
AmpliTaq (5 U/µl)	0.05 µl
dH$_2$O	3.0 µl
Total volume	10.0 µl

The PCR was carried out as follows: 1 cycle of "95°C for 3 min, 40°C for 5 min and 72°C for 5 min"; subsequently 30 cycles of "94°C for 15 sec, 40°C for 2 min and 72°C for 1 min"; after these cycles, 72°C for 5 min; and then continuously at 4°C.

Reactions were conducted using 287 primer pairs: *i.e.*, anchor primers GT15A (SEQ ID NO: 3), GT15C (SEQ ID NO: 4) and GT15G (SEQ ID NO: 5) were used in combination with arbitrary primers AG 1 to AG 110, AG 111 to AG 199 and AG 200 to AG 287, respectively. As for the arbitrary primers, oligomers having 10 nucleotides with a GC content of 50% were designed and synthesized.

For gel electrophoresis, a 6% denaturing polyacrylamide gel was prepared, and 2.5 µl sample from the PCR was applied and electrophoresed under 40 W for 210 min. After electrophoresis, the gel was scanned via a Hitachi fluorescence imaging analyzer FMBIO II, wherein the image of the gel was obtained by detecting fluorescence.

Samples from both the exacerbation stage and the remission stage were phoresed simultaneously. Gene bands shifted in the same direction in most patients, showing altered expressions, were visually determined, excised, and subjected to TA cloning and sequence determination. As a result, a DNA sequence (DD analysis band ID 2250-01; hereinafter this gene is referred to as "2250-01") whose expression is significantly enhanced in the remission stage than in

the exacerbation stage was found. The primer set used for amplifying band ID 2250-01 is shown below.

Band ID: 2250-01

Length of fragment: 421 bp (excluding the nucleotide sequence of the primer)

Anchor primer: GT15C

Name of arbitrary primer: AG00164

Sequence of arbitrary primer: CATTCTCAGG (SEQ ID NO: 6)

(3) Expression Analysis

In order to confirm the expression level of the 2250-01 gene quantitatively, quantitative PCR was further performed by ABI 7700 using the same clinical sample. Primers and TaqMan probe used for measurement by ABI 7700 were designed using Primer Express (PE Biosystems) from the sequence information obtained by the differential display method. The 5'-end and the 3'-end of TaqMan probe were labeled with FAM (6-carboxy-fluorescein) and TAMRA (6-carboxy-N,N,N',N'-tetramethylrhodamine), respectively.

2250-01 forward primer:

TGCCTTGTCTAGAACTGCACAG (SEQ ID NO: 7)

2250-01 reverse primer:

AAGTGTGTTGGACCAAGCAGC (SEQ ID NO: 8)

2250-01 TaqMan probe:

AAGTCAGTGCAGAGCCTGGATGAGGA (SEQ ID NO: 9)

cDNA was used as a template that was prepared by reverse transcription from the total RNA using poly-T (12 to 18 mer) as primers. In order to make a standard curve for the calculation of copy numbers, a plasmid clone containing the nucleotide sequence amplified using both primers was prepared, and serial dilutions thereof were utilized as the template for the reaction. The reaction mixture composition for monitoring PCR amplification is shown in Table 4.

Table 4

Reaction mixture composition for ABI-PRISM 7700 (amount per well)

Sterile distilled water	25.66 (μl)
10x TaqMan buffer A	5
25 mM MgCl$_2$	7
dATP (10 mM)	1.2
dCTP (10 mM)	1.2
dGTP (10 mM)	1.2
dUTP (10 mM)	1.2
Forward Primer (100 μM)	0.15
Reverse Primer (100 μM)	0.15
TaqMan Probe (6.7 μM)	1.49
AmpliTaq Gold (5 U/μl)	0.25
AmpErase UNG (1 U/μl)	0.5
Template solution	5
Total volume	50

5   In order to correct the difference in the cDNA concentrations
among the samples, the same quantitative analysis was carried out
for the β-actin gene that was used as internal standard, and, by
performing correction based on its copy number, the copy number of
the target gene was calculated.   For the quantification of the β-actin
10   gene, the human cDNA was used as a template.

As the primers and probe for the measurement of β-actin were
used those attached to TaqMan β-actin Control Reagents (PE Biosystems).
Their nucleotide sequences are shown below.   The "2250-01" expression
levels (copy/ng RNA) corrected for that of β-actin are shown in Table
15   5 and Fig. 1.

β-Actin forward primer:
TCA CCC ACA CTG TGC CCA TCT ACG A (SEQ ID NO: 10)
β-Actin reverse primer:
CAG CGG AAC CGC TCA TTG CCA ATG G (SEQ ID NO: 11)
20   β-actin TaqMan probe:
5'-(FAM)ATGCCC-T(TAMRA)-CCCCCATGCCATCCTGCGTp-3'   (SEQ ID NO: 12)
FAM: 6-carboxy-fluorescein:

TAMRA: 6-carboxy-N,N,N',N'-tetramethylrhodamine

Table 5

2250-01 gene expression level (copy/ng RNA)

Patient No.	Exacerbation stage	Remission stage
1	454.19	5298.42
2	137.06	167.13
3	53.86	4543.94
4	1577.46	642.43
5	403.84	4655.96
6	3745.25	801.14
7	173.98	286.83

(4) Statistical analysis

Using the above-mentioned data, parametric multiple comparison test and non-parametric multiple comparison test were carried out. Four out of the seven above-mentioned atopic dermatitis patients (patient numbers 1, 2, 3 and 5) showed remarkable decrease in the number of eosinophils associated with the transition to the remission stage due to therapy. The number of eosinophils in the blood is a useful clinical index for atopic dermatitis. Therefore, the four patient samples in which the number of eosinophils showed remarkable decrease with the transition to the remission stage (patient numbers 1, 2, 3 and 5) (n=4) were further statistically analyzed. The statistical analyses were carried out using SAS Pre-clinical Package of The SAS SYSTEM, Version 4.0 (SAS Institute Inc.). The results are shown in Table 6.

Table 6

2250-01 expression level (copy/ng RNA)

Paired test between two groups		Paired-t-test between two eosinophil-decreasing groups (n=4)
t-test Parametric	Wilcoxon test Non-parametric	
E<R p=0.274	E<R p=0.2969	E<R p=0.0572

As a result, remarkable increase in the expression of 2250-01 was observed in three out of the seven atopic dermatitis patients mentioned above (patient numbers 1, 3 and 5). These three patients were found amongst the four patients (see Table 2) whose number of eosinophils remarkably decreased with the transition to the remission stage due to therapy. The number of eosinophils in the blood is a useful clinical index for atopic dermatitis. Therefore, the changes in the expression level of 2250-01 in the 4 patient samples in which the number of eosinophils showed a remarkable decrease with the transition to the remission stage (patient numbers 1, 2, 3 and 5) (n=4) was statistically analyzed. The statistical analyses were carried out using SAS Pre-clinical Package of The SAS SYSTEM, Version 4.0 (SAS Institute Inc.).

The results confirmed significant increase in the expression in the remission stage compared to that in the exacerbation stage (p=0.0572). On the other hand, a remarkable decrease in the expression was observed in patients who did not show a decrease in eosinophil counts (patient numbers 4 and 6). These findings indicate that the expression of 2250-01 increases in association with the decrease in the number of eosinophils in the remission stage of atopic dermatitis.

EXAMPLE 2
Expression of 2250-01 in various blood cells

The expression of 2250-01 was examined in cells separated from peripheral blood collected from five normal healthy subjects. Separation of eosinophils (E) was carried out as described above. After the elution of eosinophils, neutrophils (N) were prepared by releasing the cells, which were trapped with CD16 antibody magnetic beads, from the magnetic field, eluting, and recovering. On the other hand, the monocyte fraction recovered in the middle layer by the Ficoll-centrifugation was separated into the fraction eluted from MACS CD3 antibody magnetic beads (mixture of M (monocyte) and B cell) and fraction trapped therein (T-cell fraction). Then, using MACS CD14 antibody magnetic beads, the eluted fraction was separated into the eluted fraction (B cell fraction) and trapped fraction (monocyte

fraction), and these three fractions were referred to as purified T cells, B cells and monocytes.

Eosinophils were solubilized using Isogen, while neutrophils, T cells, B cells and monocytes were solubilized with RNeasy (Qiagen), and total RNA were extracted, treated with DNase (by the same methods as described above), and subjected to the gene expression analysis. Primers, probes and others used were the same as above. Average expression levels (AVERAGE: copy/ng (corrected value)) in these blood cells are shown below.

eosinophil (E): 960

neutrophil (N): 73

basophil (B) : 36

T-cell (T): 11

monocyte (M): 103

This result indicates that 2250-01 is specifically expressed in eosinophils.

EXAMPLE 3

Extension of nucleotide sequence

Nucleotide sequence analysis was performed on 2250-01 by the 5' Rapid Amplification of cDNA Ends (5'RACE) method based on the nucleotide sequence determined in Example 1. Furthermore, cloning was performed by plaque hybridization using phage cDNA library prepared from peripheral blood eosinophil RNAs by SMART cDNA Library Construction kit (CLONTECH). Amplification was performed using primers 2250-01F and 2250-01R, which were designed within the sequence of 2250-01, and a plasmid including the sequence of 2250-01 as a template. The resulting 259-bp PCR product was purified and then used as a probe. As a result, a clone carrying an approximately 2-kb insert including the sequence of 2250-01 was obtained. The determined 2087-bp nucleotide sequence is shown in SEQ ID NO: 15. According to a genome database analysis, this sequence was found to correspond to the intronic region of a nuclear orphan receptor called NOR-1(MINOR).

·Primer sequences:

2250-01F: GTTCCAGGCAATAACATCATACC (SEQ ID NO: 13)

2250-01R: GCTACTTGTGAAACTCCCAAATG (SEQ ID NO: 14)

EXAMPLE 4
Expression analysis of the NOR-1 gene

Expression measurement by ABI7700 was performed for the reported exon sequence of NOR-1(MINOR). The primers and probe used for the TaqMan method were as follows:

Primer 1 (5'): TGGGTGCCCTGGTAGAACT (SEQ ID NO: 16);

Primer 2 (3'): GCTTCAGGTAGAAGATGCGCT (SEQ ID NO: 17); and

TaqMan probe: AGGAAGATCTGCACCCTGGGCCTC (SEQ ID NO: 18).

Similarly, to the measured result obtained using the intronic region, the tendency that the expression of the NOR-1 gene is induced in the remission stage of patients who showed notable effect against treatment with a decrease of eosinophils was reproduced (Fig. 2).

Enhancement of such gene that indicates apoptotic character in peripheral blood eosinophils during the remission stage due to therapy of atopic dermatitis, corresponds well to the decrease in the number of peripheral blood eosinophils. Thus, the expression induction of this gene seem to correlate well with therapeutic effects.

EXAMPLE 5
Search of NOR-1 receptor ligands

Promotion of a pathway that specifically leads eosinophils to cell death through the enhancement of the function of NOR-1(MINOR), very likely leads to therapy of not only asthma, but also various allergic diseases including atopic dermatitis, which was investigated by the present inventors. NOR-1(MINOR) is structurally a nuclear receptor; however, it is an orphan receptor and its native ligands and activators are so far unknown. When ligands and activators are found for NOR-1(MINOR), it can be directly activated in eosinophil cells to promote apoptosis. Therefore, ligand activators of NOR-1 are considered very likely to serve as antiallergic agents, and thus a high throughput system for ligand screening was constructed.

Mammalian Two Hybrid system was slightly modified. Specifically, as illustrated in Fig. 3, the ligand binding domain sequence or the full-length gene of NOR-1(MINOR) was inserted into

pBIND (Fig. 4) to express a protein wherein NOR-1 is fused in-frame with the DNA binding domain of GAL4. Different from other subfamilies (α and β), the AF1 region close to the N-terminus had been inferred to be important for the transcriptional activation of NOR-1(MINOR),

5   and the insertion of not only the ligand binding domain but the full-length gene was found to be necessary in the Mammalian Hybrid system. Actually, activity could not be detected for lipid-soluble metabolites existing on the metabolic map of mammals that might be natural ligands of NOR-1 in a screening system wherein only the

10   ligand-binding domain was inserted. A plasmid wherein the full-length gene of NOR-1(MINOR) had been inserted into pBIND and a luciferase reporter plasmid containing a GAL4 binding site were cotransfected into NIH3T3 cells, and the luciferase activity was measured automatically. By further addition of small molecule

15   substances to this system enables screening of ligands by their transcription enhancing activity.

NOR-1(MINOR) expression is induced under conditions where the leukocytes are hyperactive, such as in atopic dermatitis peripheral blood, and apoptosis of cells is very likely to occur as a result

20   of this induced NOR-1(MINOR) expression. Ligands existing *in vivo* may exist at sites where nuclear receptors are highly expressed. Therefore, small-molecule lipid-soluble mediators that are expected to be produced under such conditions were added to the assay system as candidate ligands to evaluate their luciferase activity enhancing

25   function. Among the lipid-soluble mediators, prostaglandins having a cyclopentenone structure, such as prostaglandin A_2, prostaglandin A_1, 15(R)-15-methyl prostaglandin A_2, 16-phenoxy tetranor prostaglandin A_2 and 17-phenyl trinor prostaglandin A_2, were found to have the function of enhancing the transcriptional activation

30   ability of NOR-1 (MINOR) (Fig. 5, Tables 7 to 13). Thus, the method established by the present inventors opened a way to discover native ligands and synthetic ligands of NOR-1 (MINOR) by high throughput. In addition, compounds such as prostaglandin A_2 and prostaglandin A_1, and metabolites related thereto were revealed to be very likely the

35   true native ligands of NOR-1 (MINOR).

Table 7

Name of compound	Structural formula	Nor1 LBD-ligand activity		Full length Nor1-ligand activity	
		RXR(+)	RXR(−)	RXR(+)	RXR(−)
Prostaglandin A$_2$		×	×	○ 10 μM	○ 10 μM
Prostaglandin A$_1$		×	×	○ 10 μM	○ 10 μM

Table 8
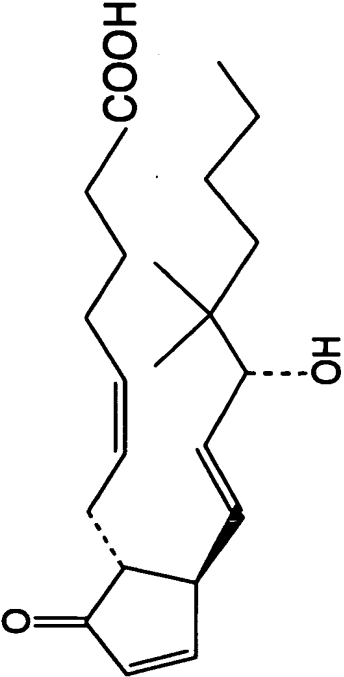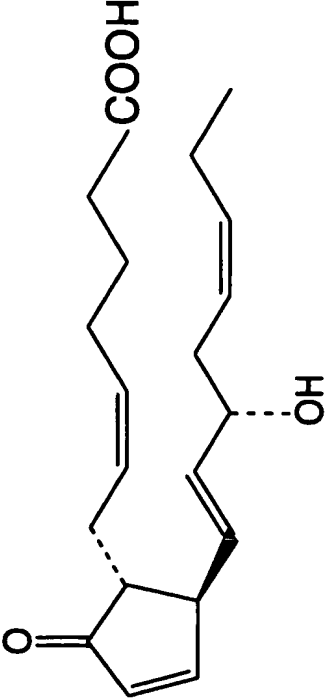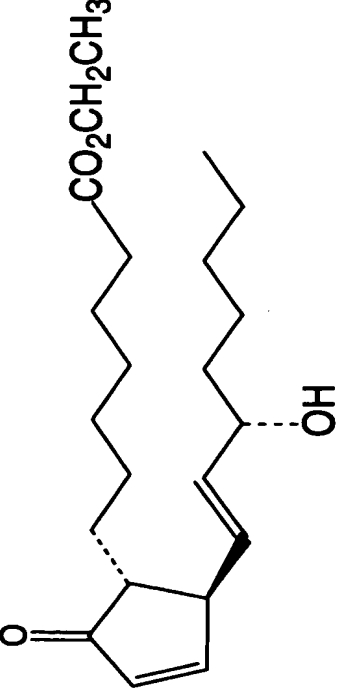
16,16-dimethyl Prostaglandin A₂	(structure, COOH / OH)	×	×	◯ 5 µM	◯ 5 µM
Prostaglandin A₃	(structure, COOH / OH)	×	×	×	×
Prostaglandin A₁ ethyl ester	(structure, $CO_2CH_2CH_3$ / OH)	×	×	×	×
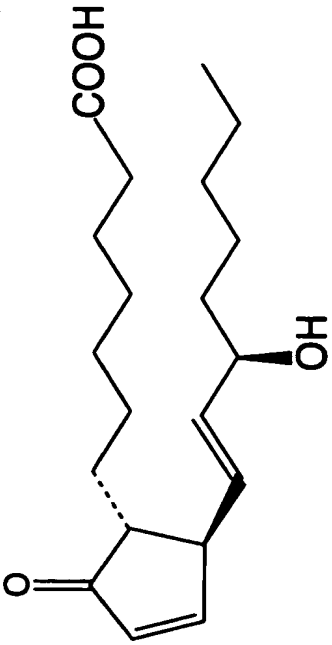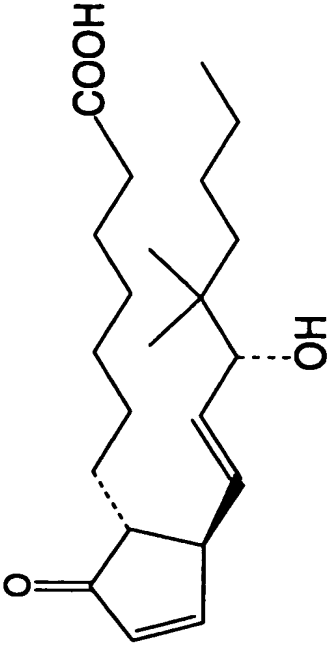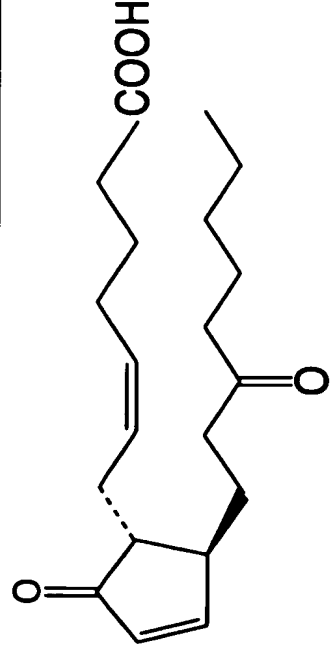
Table 9

	15-epi Prostaglandin A$_1$	16,16-dimethyl Prostaglandin A$_1$	13,14-dihydro-15-keto Prostaglandin A$_2$
	×	×	×
	×	×	×
	×	×	×
	×	×	×

Table 10
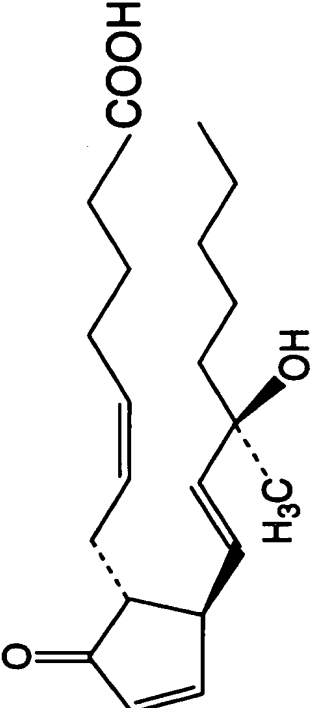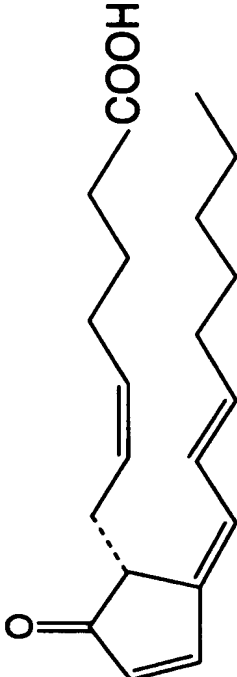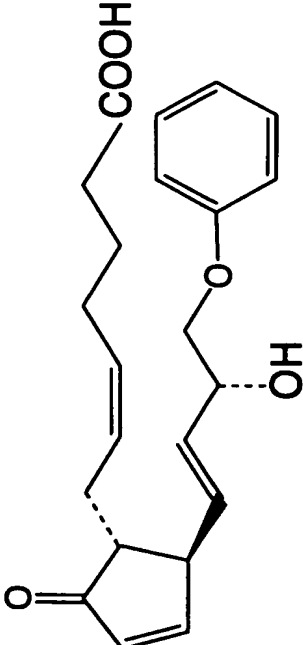
Compound	Structure				
15(R)-15-methyl Prostaglandin A$_2$	(structure)	×	×	○ 10 μM	○ 10 μM
15-deoxy-Δ^{12,14}-Prostaglandin A$_2$	(structure)	×	×	×	×
16-phenoxy tetranor Prostaglandin A$_2$	(structure)	×	×	○ 30 μM	○ 30 μM
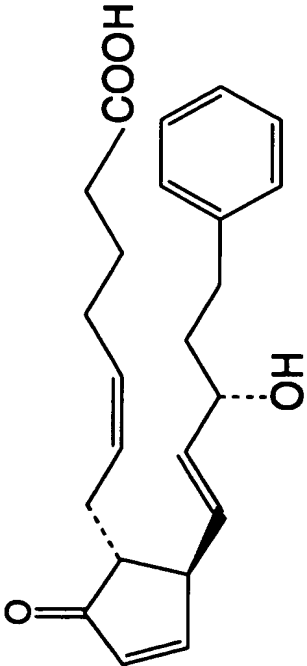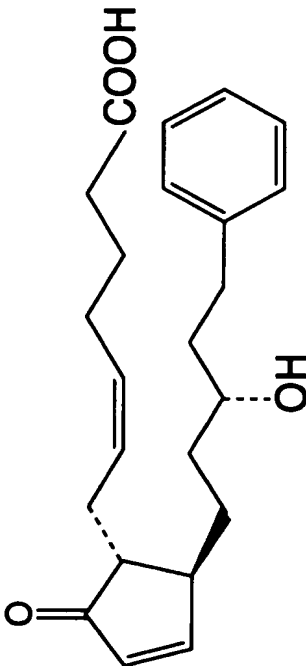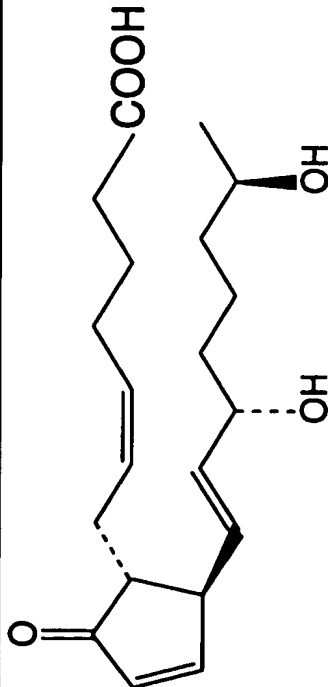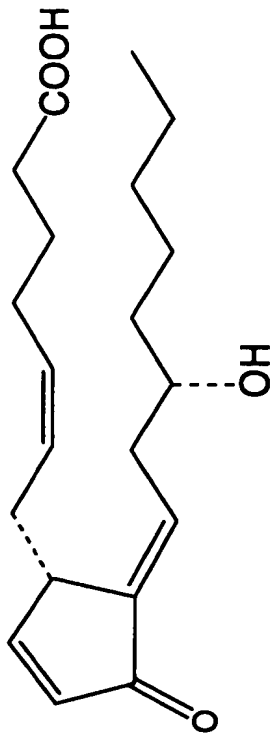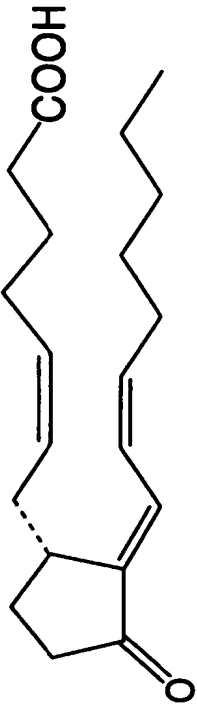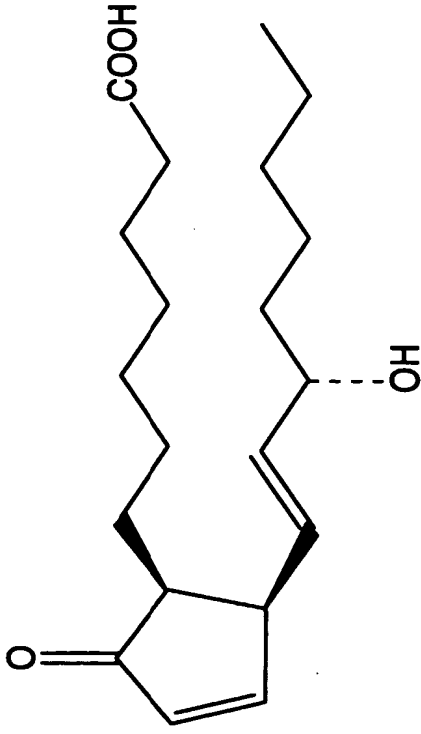
Table 11

17-phenyl trinor Prostaglandin A₂	×	×	O 10μM	O 10μM
17-phenyl trinor-13,14-dihydro Prostaglandin A₂	×	×	×	×
19(R)-hydroxy Prostaglandin A₂	×	×	×	×

Table 12

Name	Structure				
15-deoxy-$\Delta^{12,14}$-Prostaglandin A_1		×	×	×	×
Prostaglandin J_2		×	×	×	×
15-deoxy-$\Delta^{12,14}$-Prostaglandin J_2		×	×	O 10μM	×

Table 13

×	×	ND
×	×	O 10 μM
×	×	ND
×	×	ND
Δ12-Prostaglandin J₂	9.10-dihydro-15-deoxy-Δ^{12,14}-Prostaglandin J₂ (CAY10410)	8-iso Prostaglandin A₁

In the Tables above, ND denotes not determined.

<u>EXAMPLE 6</u>

<u>Virtual compounds</u>

The binding position of the PGA derivatives to the Nor1 ligand
binding domain was simulated by the pharmacophore model (Fig. 6),
and based on the information on structure activity relationship in
the reporter system of the PGA derivatives, compounds other than the
PGA derivatives that fit similarly into the binding pocket were
selected from the Catalyst database (screened from BioByte Master
File 2001 39,383 compounds, 2,198,646 conformations). The list
(including the structural formula) of 155 compounds selected as
strongly binding compounds by the simulation are shown in Tables 14
to 49, and the list of 281 compounds selected subsequently are shown
in Tables 50 to 58.

Table 14
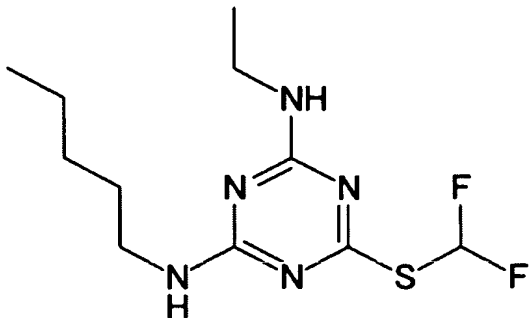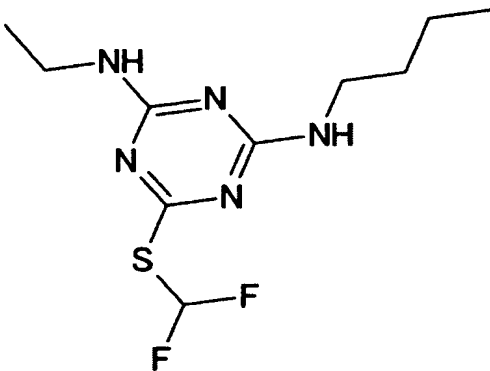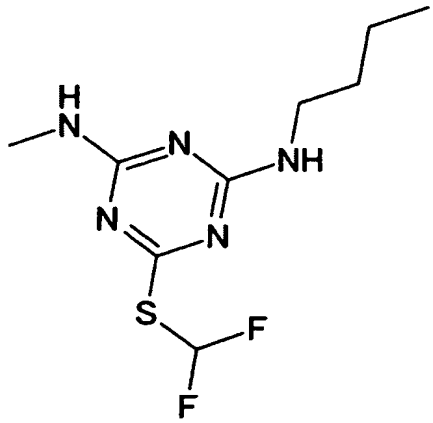
Name of compound
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 135TRIAZINE2DIFLUOROMETHIO4BUTYLAMINO6ETHYLAMINO
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Table 15
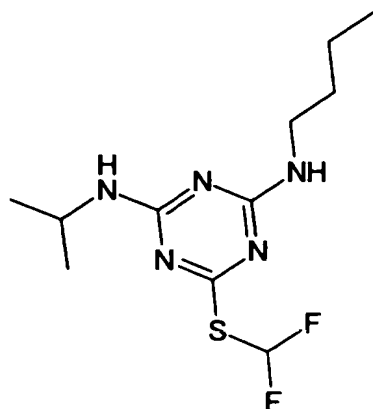
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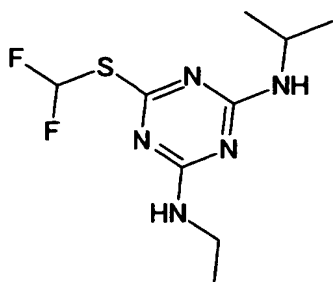
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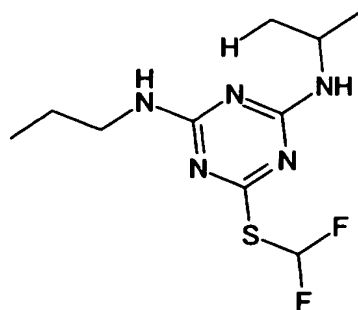
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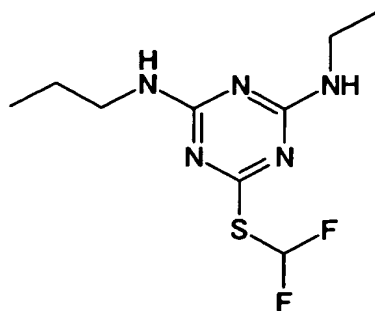
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Table 16
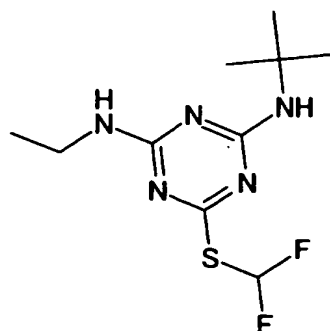
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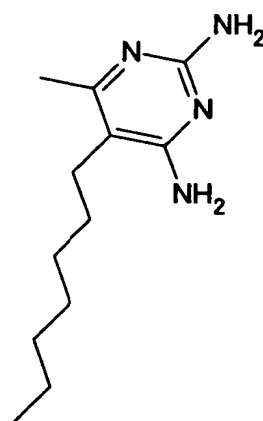
24DIAMINO5HEPTYL6MEPYRIMIDINE
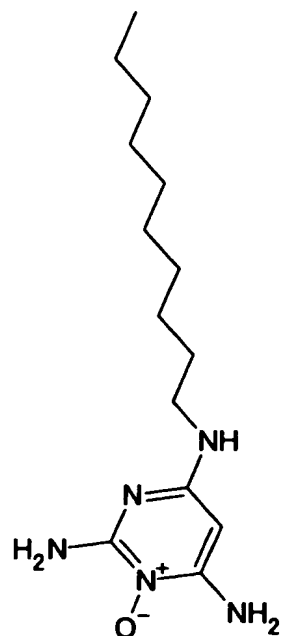
24DIAMINO6DECYLAMINOPYRIMIDINE3OXIDE

Table 17
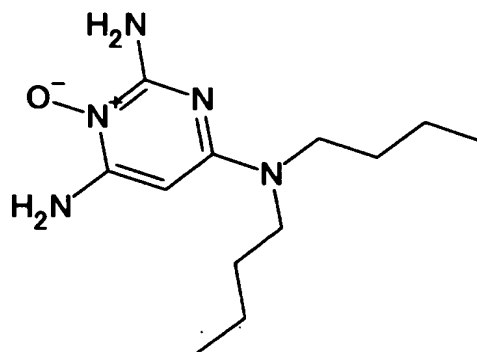
24DIAMINO6DIBUTYLAMINOPYRIMIDINE3OXIDE
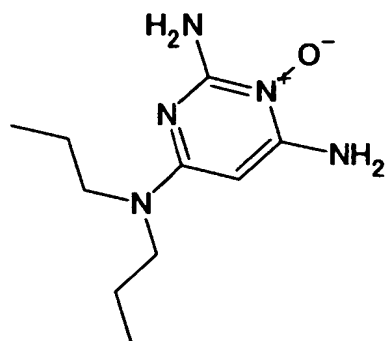
24DIAMINO6DIPROPYLAMINOPYRIMIDINE3OXIDE
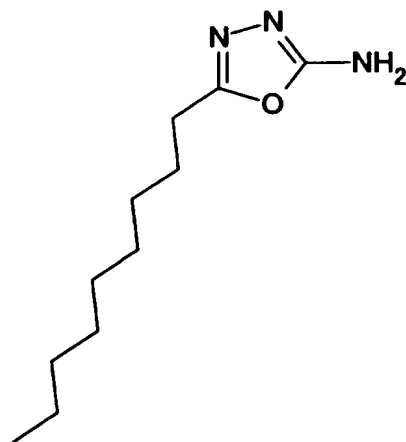
2AMINO5NONYL134OXADIAZOLE

Table 18
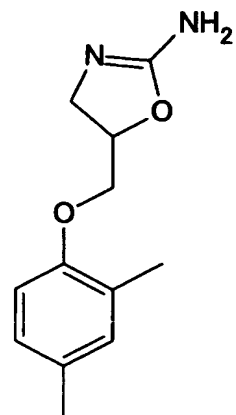
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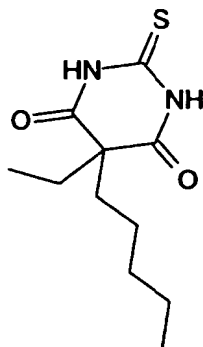
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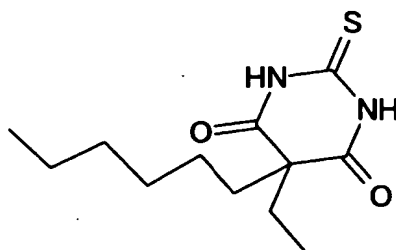
2THIO5ET5HEXYLBARBITURICACID
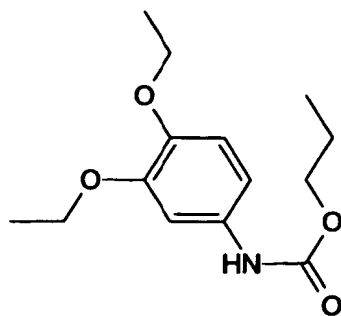
34DIETHOXYPHENYLCARBAMATEOPROPYL

Table 19
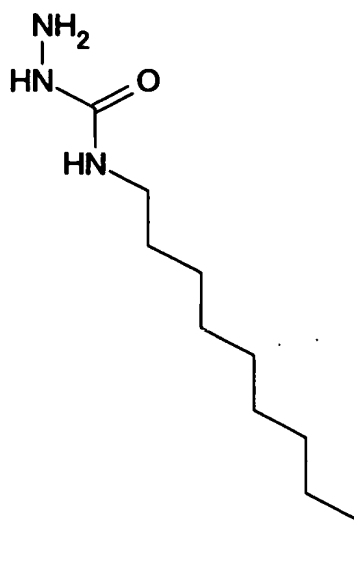
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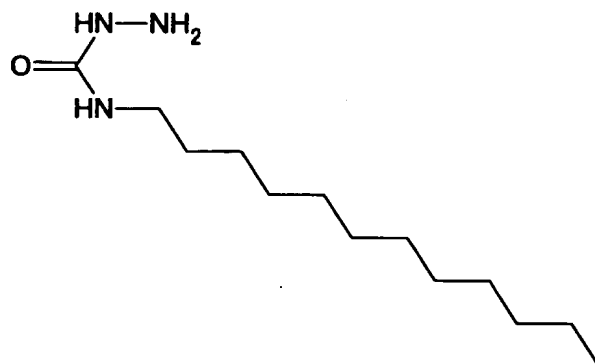
4DODECYLSEMICARBAZIDE
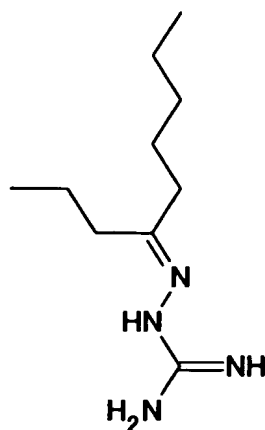
4NONANONEGUANYLHYDRAZONE

Table 20
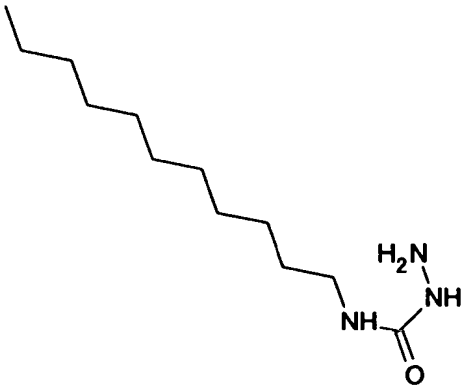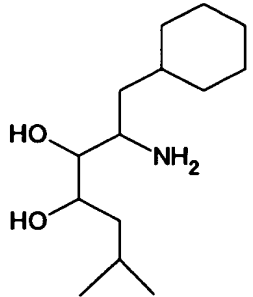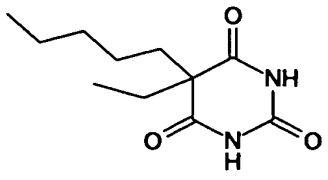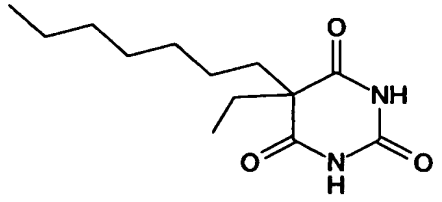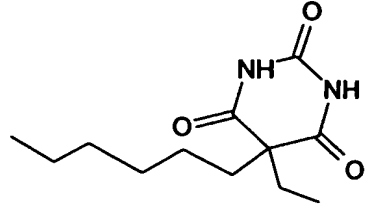
4UNDECYLSEMICARBAZIDE

ACDMH

BARBITURICACID5AMYL5ETHYL

BARBITURICACID5HEPTYL5ETHYL

BARBITURICACID5HEXYL5ETHYL

Table 21
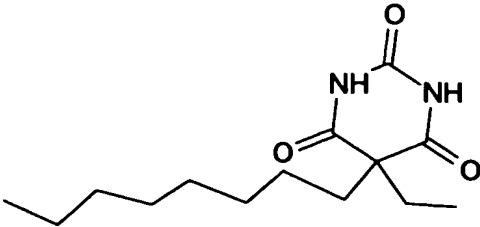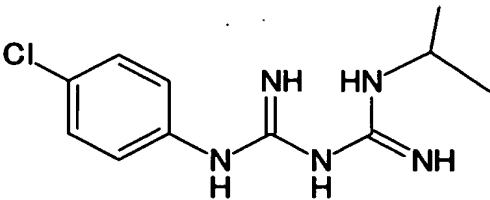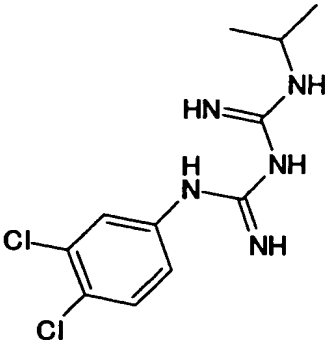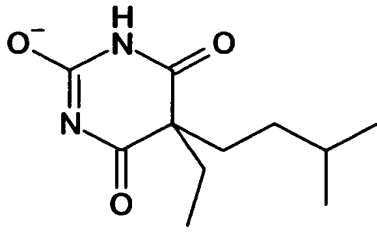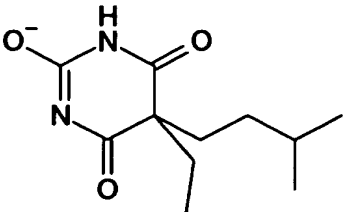
BARBITURICACID5OCTYL5ETHYL

CHLOROGUANIDE

CHLORPROGUANIL

DIETPROTRIPTYLINESALTAMOBARBITAL

DIMEPROTRIPTYLINESALTAMOBARBITAL

Table 22
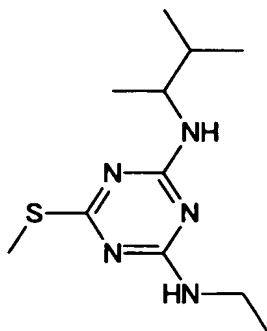
DIMETHAMETRYN
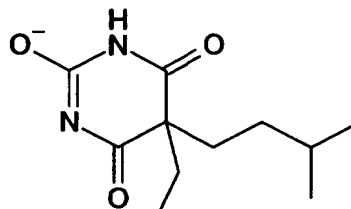
DIPRPROTRIPTYLINESALTAMOBARBITAL
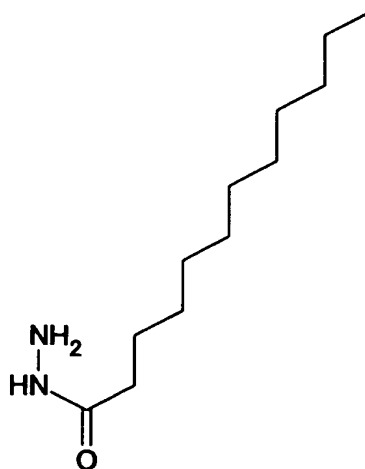
DODECANOICACIDHYDRAZIDE
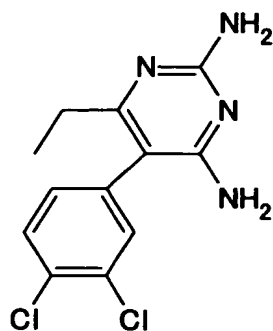
ETOPRINE

Table 23
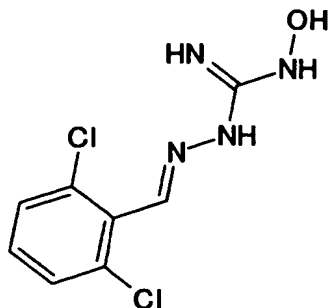
GUANOXABENZ
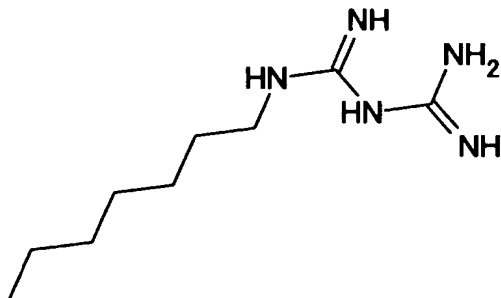
HEPTYLBIGUANIDE
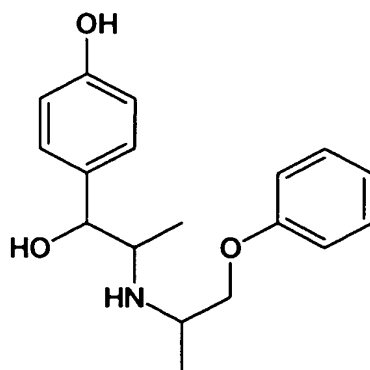
ISOXSUPRINE
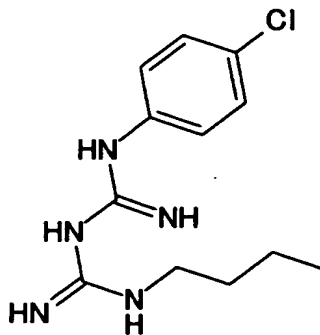
N1PCHLOROPHENYLN5BUTYLBIGUANIDE

Table 24
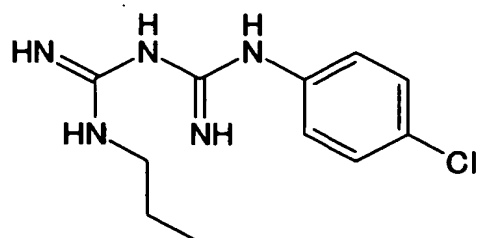
N1PCHLOROPHENYLN5PROPYLBIGUANIDE
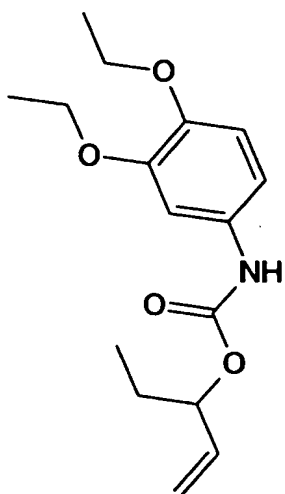
N34DIETHOXYPHENYLCARBAMATE1PENTEN3YLESTER
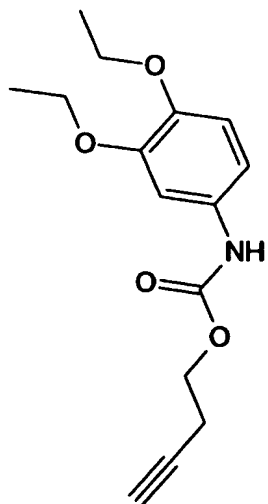
N34DIETHOXYPHENYLCARBAMATE4BUTYNYLESTER

Table 25
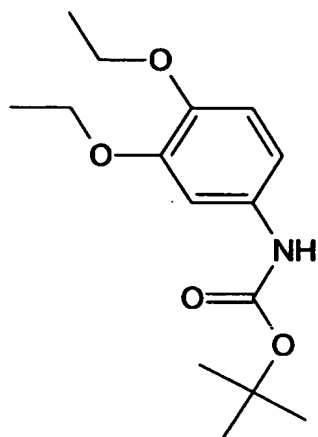
N34DIETHOXYPHENYLCARBAMATETBUTYLESTER
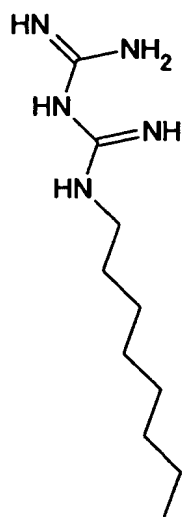
OCTYLBIGUANIDE
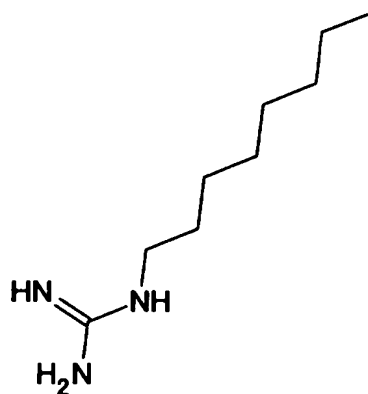
OCTYLGUANIDIUMSULFATE

Table 26
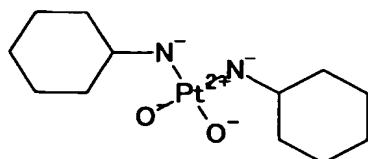
PLATINUMBISCYCLOHEXYLAMMONIODIAQUADINITRATE
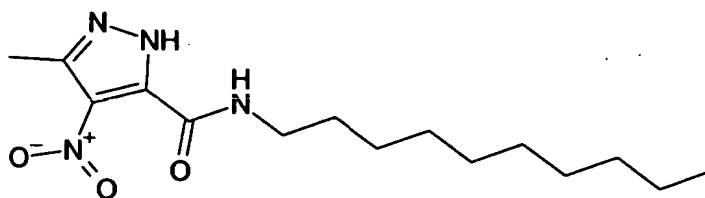
PYRAZOLE3METHYL4NITRO5NDECYLCARBOXAMIDO
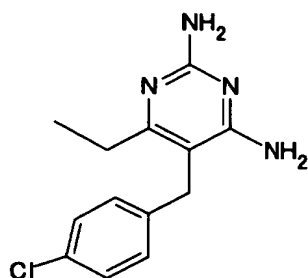
PYRIMIDINE24DIAMINO5PCHLOROBENZYL6ETHYL
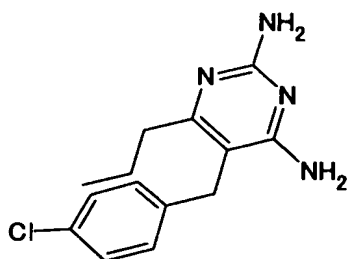
PYRIMIDINE24DIAMINO5PCHLOROBENZYL6PROPYL
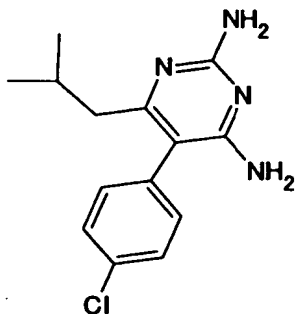
PYRIMIDINE24DIAMINO5PCHLOROPHENYL6IBUTYL

Table 27
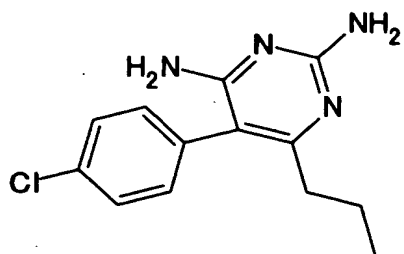
PYRIMIDINE24DIAMINO5PCHLOROPHENYL6PROPYL
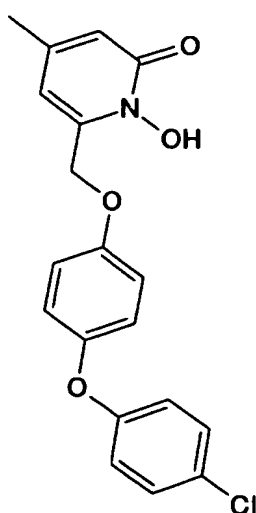
RILOPIROX
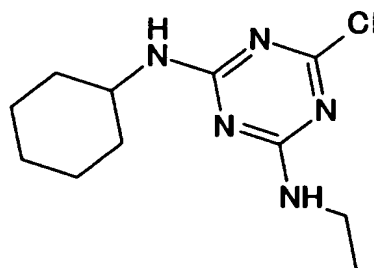
SIMAZINE2CYCLOHEXYLAMINEANALOG
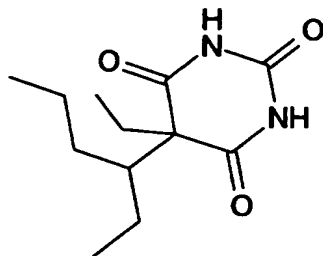
TETRABARBITAL

Table 28
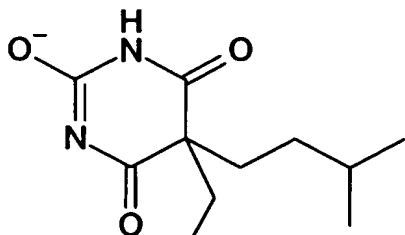
TETRABUTYLAMMONIUMAMOBARBITURATE
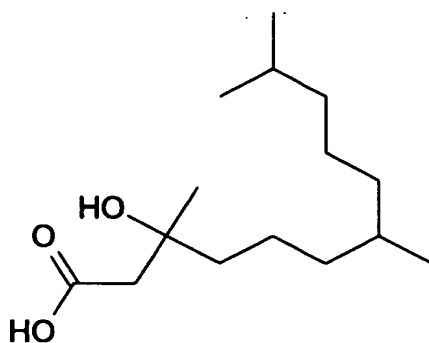
TRETHOCANOICACID
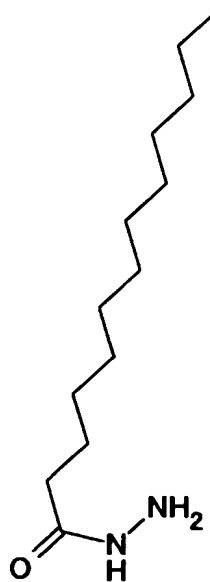
TRIDECANOICACIDHYDRAZIDE

Table 29
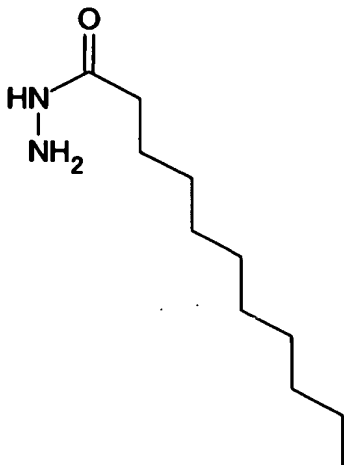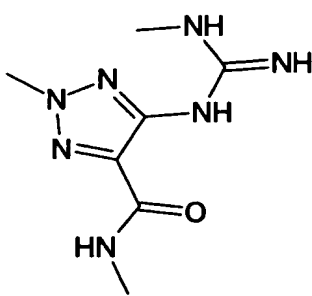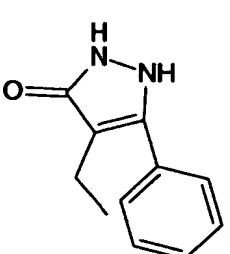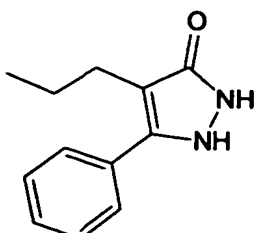
UNDECANOICACIDHYDRAZIDE

123TRIAZOLE2METHYL4METHYLCARBAMOYL53METHYLGUANIDINO

12DIHYDROPYRAZOLONE4ETHYL5PHENYL

12DIHYDROPYRAZOLONE4PROPYL5PHENYL

Table 30
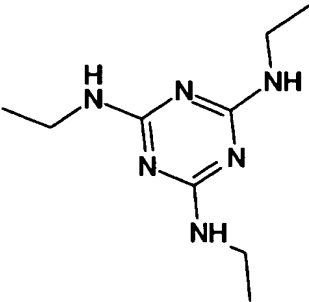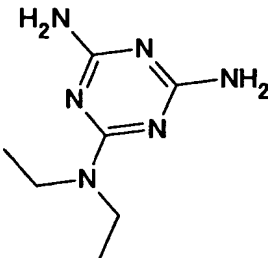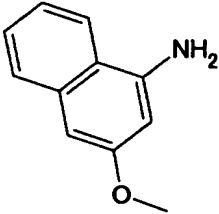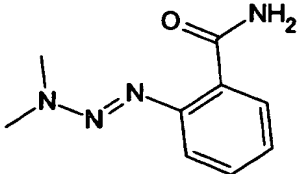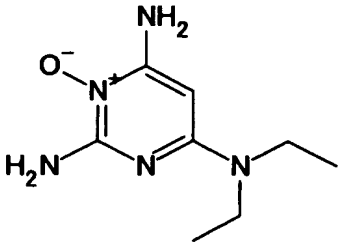
135TRIAZINE246TRISETHYLAMINO

135TRIAZINE24DIAMINO6DIETHYLAMINO

1NAPHTHALENEAMINE3METHOXY

233DIMETHYL1TRIAZINOBENZAMIDE

24DIAMINO6DIETHYLAMINOPYRIMIDINE3OXIDE

Table 31
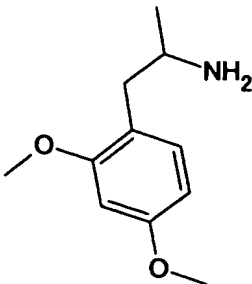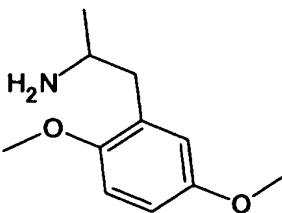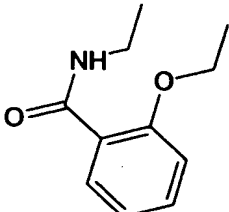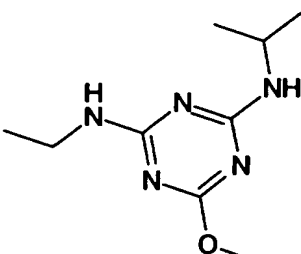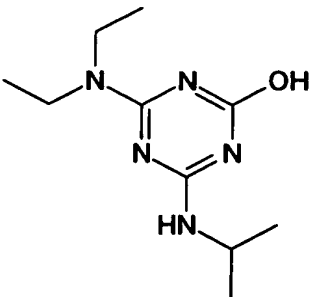
24DIMETHOXYAMPHETAMINE

25DIMETHOXYAMPHETAMINE

2ETHOXYBENZAMIDENETHYL

2METHOXY4ETAMINO6IPROPYLAMINOSTRIAZINE

2OH4IPROPYLAMINO6DIETAMINOSTRIAZINE
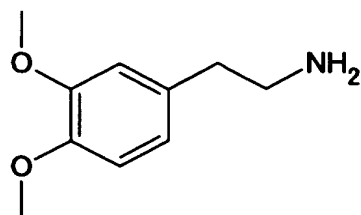
Table 32

34DIMETHOXYPHENETHYLAMINE
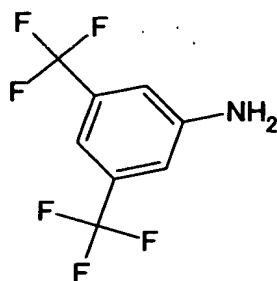
35BISTRIFLUOROMETHYLANILINE
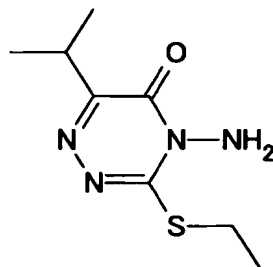
3ETHYLTHIO4AMINO6IPR124TRIAZINE5ONE
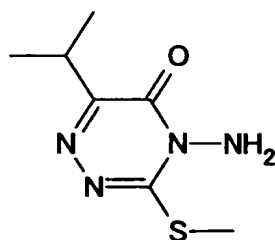
3METHIO4AMINO6IPR124TRIAZINE5ONE
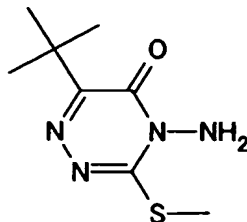
3METHIO4AMINO6TBU124TRIAZINE5ONE

Table 33
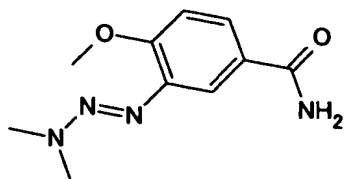
4METHOXY333DIMETHYLTRIAZENOBENZAMIDE
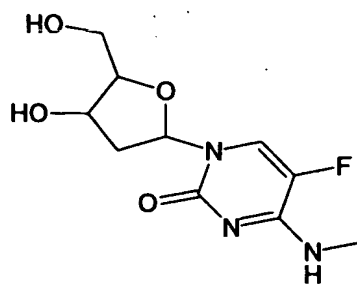
5FLUORONMETHYL2DEOXYCYTIDINE
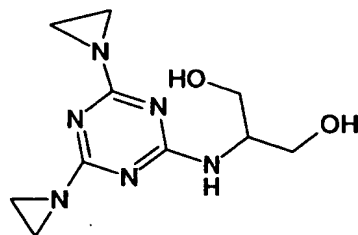
6BISHOCH2MEAMINO24DIAZIRIDINYLTRIAZINE
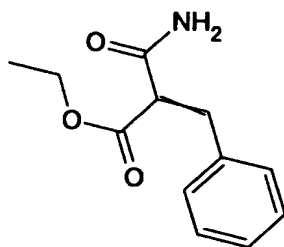
AAMIDOETHYLCINNAMATE
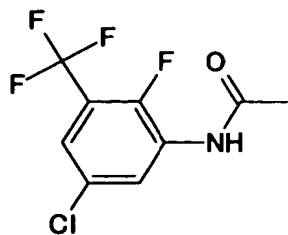
ACETANILIDE2FLUORO3TRIFLUOROMETHYL5CHLORO

Table 34
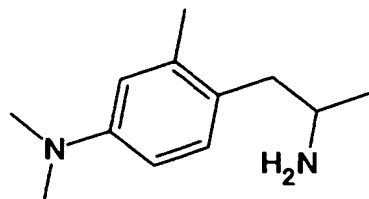
AMIFLAMINE
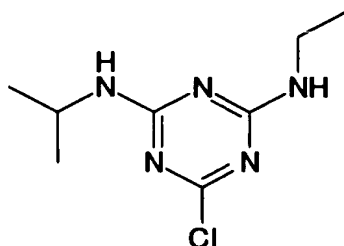
ATRAZINE
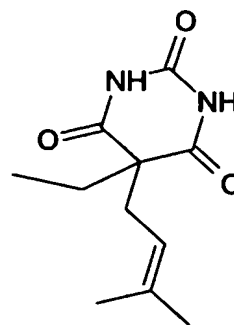
BARBITURICACID5ETHYL53METHYLBUTEN2YL
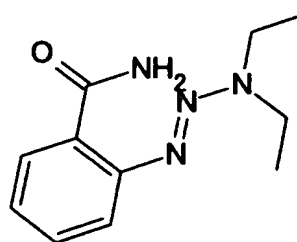
BENZAMIDE233DIETHYLTRIAZENYL
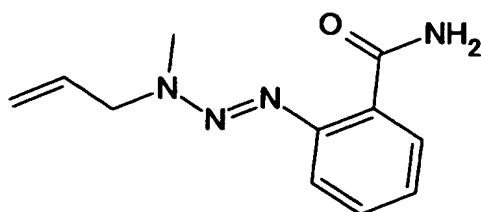
BENZAMIDE23METHYL3ALLYLTRIAZENYL

Table 35
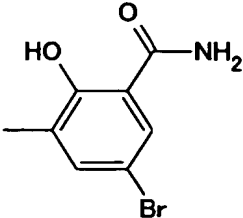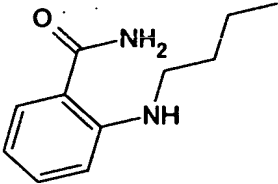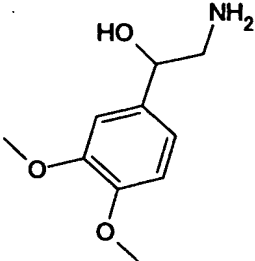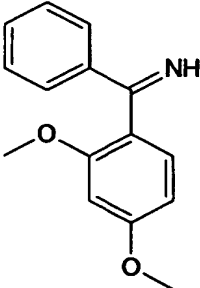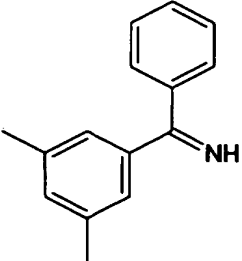
BENZAMIDE2HYDROXY3METHYL5BROMO

BENZAMIDEOBUTYLAMINO

BENZENEETHANEAMINEAHYDROXY34DIMETHOXY

BENZENEMETHANIMINE24DIMETHOXYAPHENYL
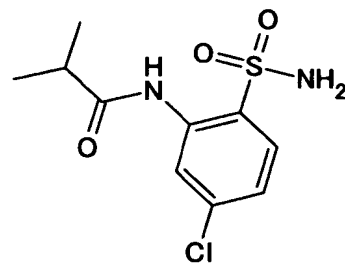

BENZENEMETHANIMINE35DIMETHYLAPHENYL
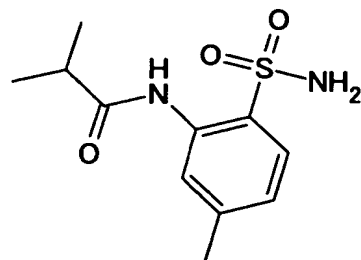
Table 36
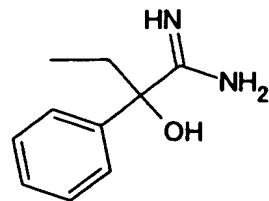
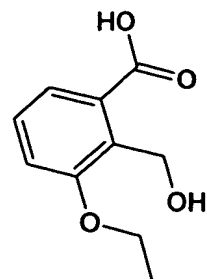BENZENEMETHANOLAETHYLAAMIDINO
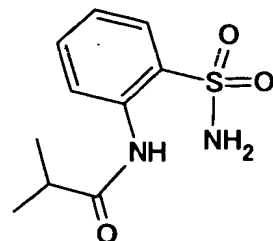
BENZENESULFONAMIDE2IBUTYROYLAMINO
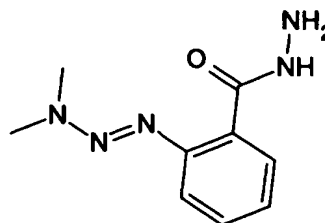

BENZENESULFONAMIDE2IBUTYROYLAMINO4CHLORO

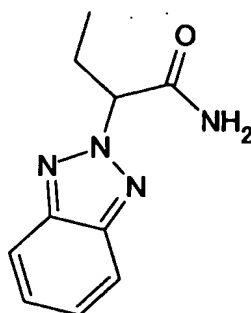BENZENESULFONAMIDE2IBUTYROYLAMINO4METHYL

BENZOICACID2HYDROXYMETHYL3ETHOXY
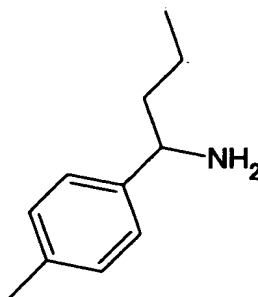
Table 37

BENZOICACIDHYDRAZIDEO33DIMETRIAZINO

BENZOTRIAZOLE22BUTYRAMIDE

BUTYLAMINE1PTOLYL
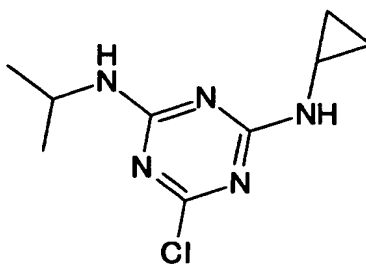
CYPRAZINE
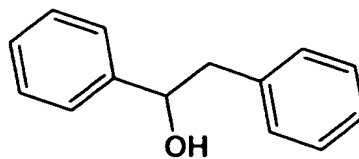
ETHANOL12DIPHENYL

Table 38
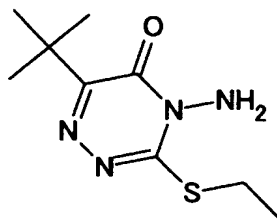
ETHIOZIN
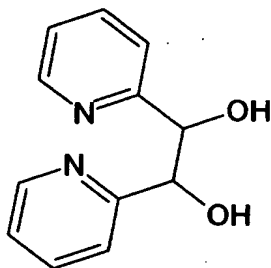
ETHYLENEGLYCOL12BIS2PYRIDYL
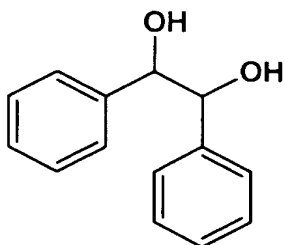
ETHYLENEGLYCOL12DIPHENYL
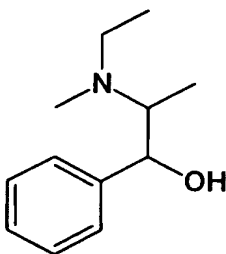
ETHYLEPHEDRINE
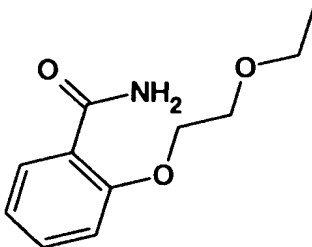
ETOSALAMIDE

Table 39
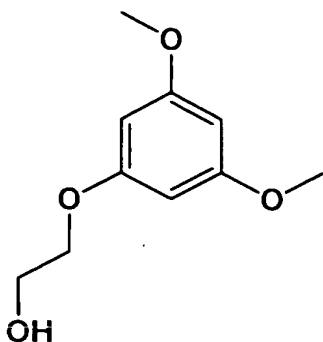
FLOVERINE
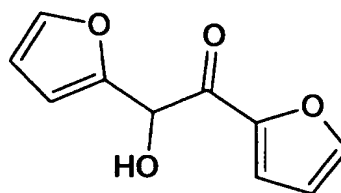
FURAN2CHOHCOFURYL
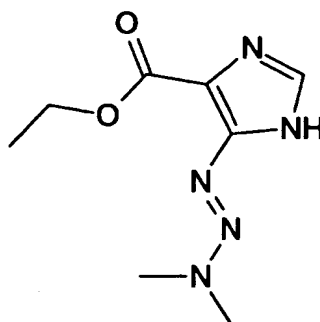
IMIDAZOLE4ETHOXYCARBONYL533DIMETHYLTRIAZENYL
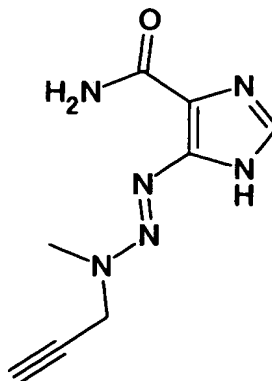
IMIDAZOLE5CARBOXAMIDE43METHYL3PROPYNYLTRIAZENYL

Table 40
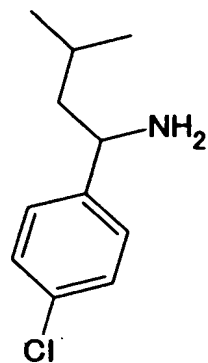
IPENTYLAMINEA4CHLOROPHENYL
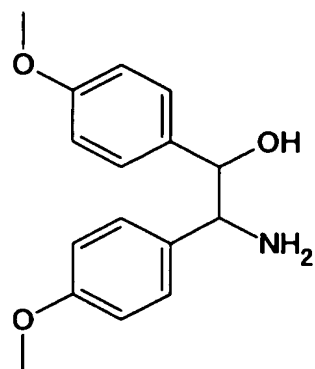
ISOLADOL
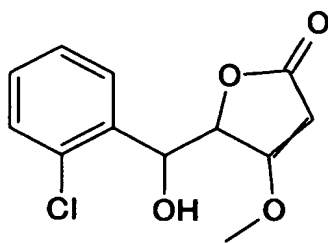
LOSIGAMONE
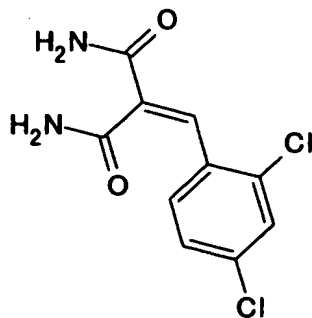
MALONAMIDE24DICHLOROBENZAL

Table 41
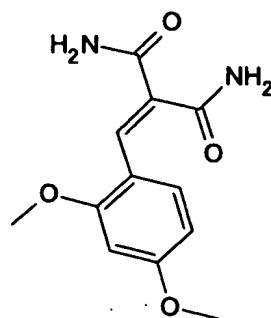
MALONAMIDE24DIMETHOXYBENZAL
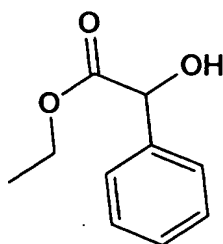
MANDELICACIDETHYLESTER
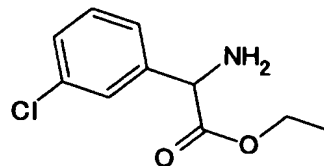
MCHLOROPHENYLGLYCINEETHYLESTER
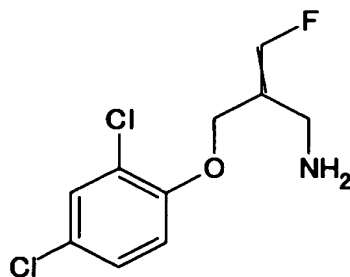
MDL72145
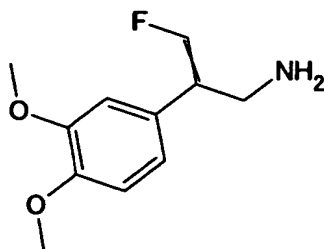
MDL72638

Table 42
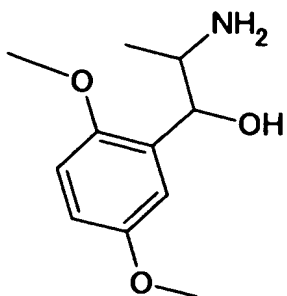
METHOXAMINE
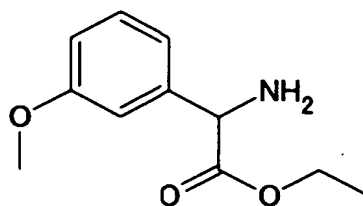
MMETHOXYPHENYLGLYCINEETHYLESTER
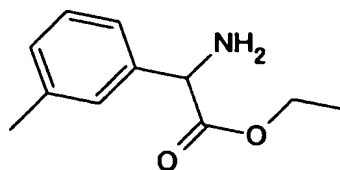
MMETHYLPHENYLGLYCINEETHYLESTER
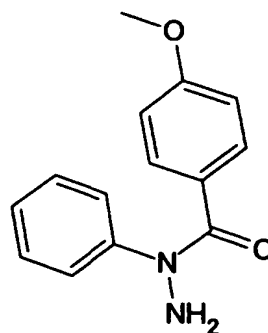
N1PHENYLN1PMETHOXYBENZOYLHYDRAZINE
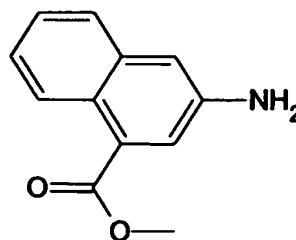
NAPHTHALENE2AMINO4METHOXYCARBONYL

Table 43
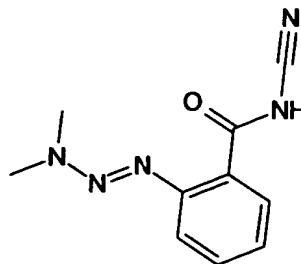
NCYANO233DIMETHYL1TRIAZENOBENZAMIDE
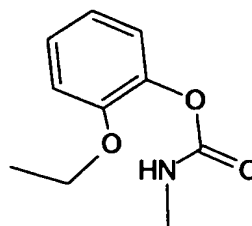
NMETHYL2ETHOXYPHENYLCARBAMATE
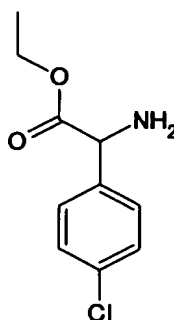
PCHLOROPHENYLGLYCINEETHYLESTER
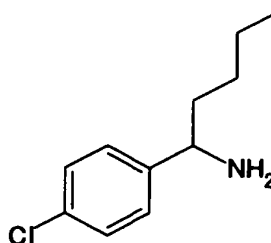
PENTYLAMINEAPCHLOROPHENYL
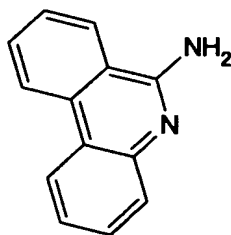
PHENANTHRIDINE6AMINO

Table 44
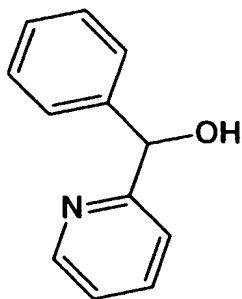
PHENYLAPYRIDYLCARBINOL
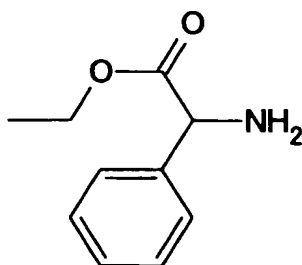
PHENYLGLYCINEETHYLESTER
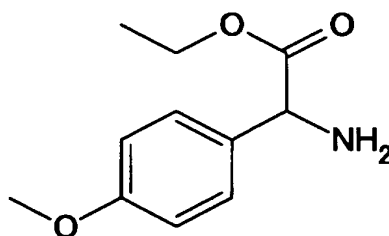
PMETHOXYPHENYLGLYCINEETHYLESTER
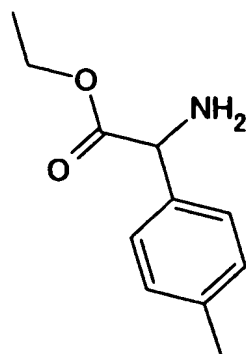
PMETHYLPHENYLGLYCINEETHYLESTER

Table 45
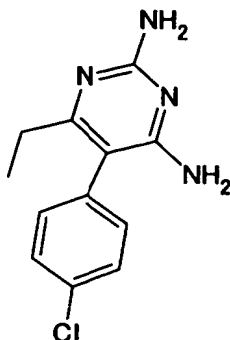
PYRIMETHAMINE
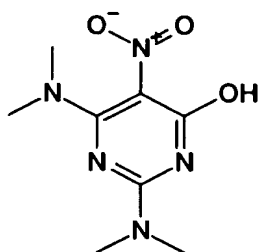
PYRIMIDINE24BISDIMETHYLAMINO6HYDROXY5NITRO
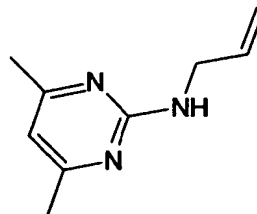
PYRIMIDINE2ALLYLAMINO46DIMETHYL
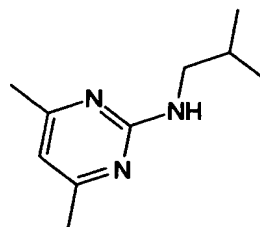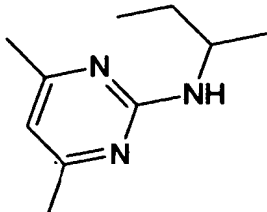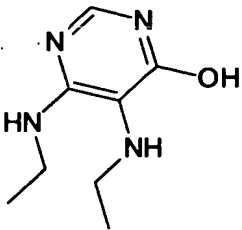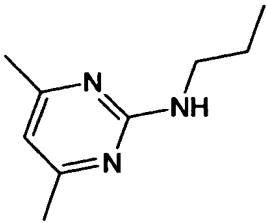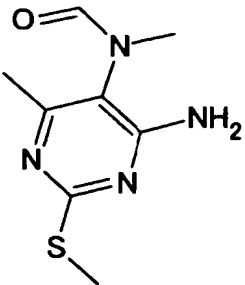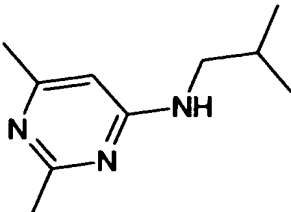
PYRIMIDINE2IBUTYLAMINO46DIMETHYL
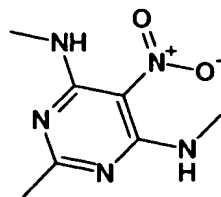
PYRIMIDINE2METHYL46BISMETHYLAMINO5NITRO

Table 46

PYRIMIDINE2SECBUTYLAMINO46DIMETHYL

PYRIMIDINE45BISETHYLAMINO6HYDROXY

PYRIMIDINE46DIMETHYL2PROPYLAMINO

PYRIMIDINE4AMINO6METHYL5METHYLFORMAMIDO2METHYLTHIO

PYRIMIDINE4IBUTYL26DIMETHYL
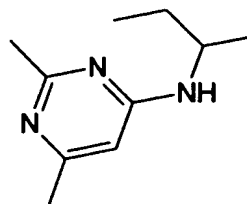
Table 47

PYRIMIDINE4SECBUTYLAMINO26DIMETHYL

PYRIMIDINE4TBUTYLAMINO26DIMETHYL

PYRIMIDINE5AMINO2METHYL46BISMETHYLAMINO

PYRIMIDINE5FORMAMIDO46BISMETHYLAMINO2METHYLTHIO

QUINOLINETRANSDECAHYDRO4METHOXYCARBONYL2METHYL

Table 48
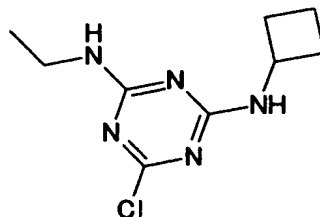
SIMAZINE2CYCLOBUTYLAMINEANALOG
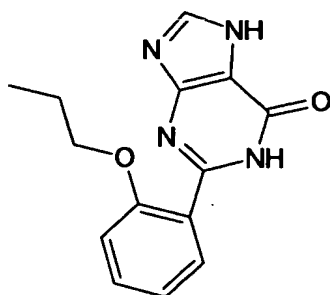
SKF96231
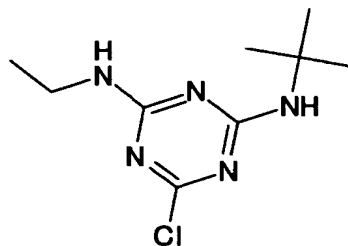
TERBUTHYLAZINE
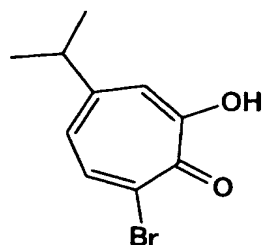
TROPALONE4IPROPYL7BROMO
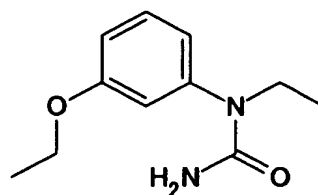
UREA1ETHYL1METHOXYPHENYL

Table 49
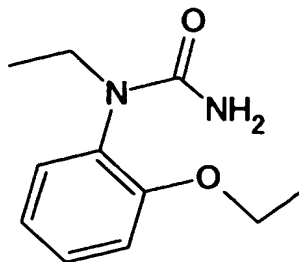
UREA1ETHYL1OETHOXYPHENYL
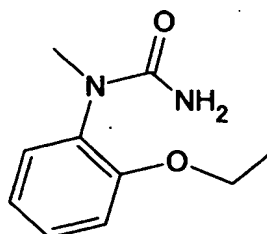
UREA1METHYL1OETHOXYPHENYL
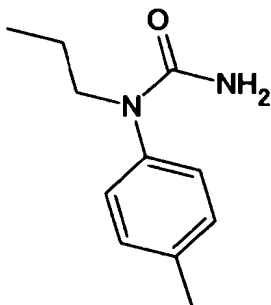
UREA1PROPYL1PTOLYL

Table 50

Name of structure	MW	LUDI_ score	HB_ score	LIPO_ score	Rotlbon ds	Rule of 5 Violations	CONS_ score	LIGscore CFF
1OUNDECEN1OL	170.2942	-323	0	0	0		4	1.49
11DIPHENYLHYDRAZINE	184.2402	-66	0	29	2	0		
1234H4ISOQUINOLINE58DIMETHOXY	193.245	-90	0	5	2	0		
123BUTANETRIONE1DIMETHYLAMINO2OXIME3METHOXIME	187.198	-95	0	0	5	0		
12DIHYDROPYRAZOLONE4BUTYL5PHENYL	216.2822	-166	0	5			5	2.2
134DIMETHOXYPHENYL2PROPANOL	196.2456	-110	0	35	5	0		
135TRIAZINE246TRIAMINENNDIETHYL	182.2278	-125	0	20	4	0		
135TRIAZINE2DIFLUOROMETHIO46BISETHYLAMINO	249.281	-188	0	8			4	1.75
135TRIAZINE2DIFLUOROMETHIO46BISMETHYLAMINO	221.2274	-145	0	0			4	1.51
135TRIAZINE2DIFLUOROMETHIO4ETHYLAMINO6METHYLAMINO	235.2542	-171	0	0			5	2.24
135TRIAZINE2DIFLUOROMETHIO4TBUTYLAMINO6METHYLAMINO	263.3078	-166	0	5			5	1.11
13PROPANEDIOL2BUTYL2ETHYL	160.2558	-236	0	11			4	1.96
1HYDROXYPENTACHLOROCYCLOHEXANE	272.3857	-66	0	29	1	0		
1NDIMETHYLHEXAHYDROFLUOREN4AAMINE	215.3376	-120	0	0	1	0		
1PHENYL13PROPANEDIOL2DICHLOROACETAMIDO	278.1346	-195	0	26			4	1.46
21HPTERIDINONE4AMINO167TRIMETHYL	205.2188	-95	0	0	0	0		
226CL24MEOPHENYLIMINOIMIDAZOLINE	260.1224	-66	0	29	2	0		
226DIETHYLPHENYLIMINOIMIDAZOLIDINE	217.3132	-128	0	17	3	0		
234TRIMETHOXYAMPHETAMINE	225.287	-104	0	41	5	0		
235TRICHLOROPHENOL	197.4481	-95	0	0	1	0		
235TRIMETHYLPHENOL	136.1932	-95	0	0	1	0		
23BENZOOCTAHYDRONAPHTHALENE34DIOH34DIAX	218.295	-63	0	32	2	0		
24DICHLOROBENZYLALCOHOL	177.0298	-118	0	2	2	0		
24DICHLROPHENYLARSONICACID	270.9313	-90	0	5	3	0		
25DIMETHOXY4METHIOAMPHETAMINE	241.3476	-127	0	44	5	0		
25DIMETHOXY4METHYLAMPHETAMINE	209.2876	-122	0	23	4	0		
26DIMETHYL4PYRIMIDINAMINE	123.1572	-95	0	0	0	0		
2ALLYLPHENOL3METHOXY	164.2036	-145	0	0			4	1.72
2AMINO2TETRAFLUOROETHYLVINYLPHENYLTHIONE	263.2524	-122	0	23	4	0		

Table 51

Name									
2AMINO4METHOXY6TRICHLORMETHYLSYMTRIAZINE	243.4797	-120	0	0	1	0		
2CHLOROETHYLN3CHLOROPHENYLCARBAMATE	234.0816	-110	0	35	5	0		
2MEO46BISIPROPYLAMINOSTRIAZINE	225.293	-122	0	23	5	0		
2METHIO4ETAMINO6IPRAMINOSTRIAZINE	227.3268	-171	0	0			4	1.92
2METHOXY4MEAMINO6IPROPYLAMINOSTRIAZINE	197.2394	-115	0	5	4	0		
2METHYL4BENZYLAMINO123BENZOTRIAZINIUMIODIDE	251.3103	-107	0	38	3	0		
2METHYLTHIOPHENYLCARBAMATENMETHYL	197.2514	-120	0	0	4	0		
2NNDIETHYLAMINO1PHENYLETHANOL	193.2882	-201	0	20			4	1.56
2OH46BISETHYLAMINOSTRIAZINE	183.2126	-125	0	20	5	0		
2OH4ETAMINO6DIETAMINOSTRIAZINE	211.2662	-171	0	0			5	1.92
2OH4ETAMINO6IPROPYLAMINOSTRIAZINE	197.2394	-122	0	23	5	0		
2PYRIDINEAMINE46DIMETHYL	122.1694	-95	0	0	0	0		
2PYRIDONE56BISMETHIOMETHYL	215.3278	-196	0	0			4	2.06
345TRIMETHOXYAMPHETAMINE	225.287	-125	0	20	5	0		
34DIMETHOXYAMPHETAMINE	195.2608	-128	0	17	4	0		
34DIMETHOXYBENZYLALCOHOL	168.192	-120	0	0	4	0		
35DIBROMOPHENOL	251.905	-95	0	0	1	0		
35DICHLOROANILINE	162.0182	-95	0	0	0	0		
35DICHLOROPHENOL	163.003	-95	0	0	1	0		
35DICLC6H3NHNCCNCOOET	286.117	-94	0	26	6	0		
35DIMETHOXY4BROMOPHENENETHYLAMINE	260.1301	-113	0	32	4	0		
35DIMETHOXYPHENOL	154.1652	-95	0	0	3	0		
35DIMETHYLANILINE	121.1816	-95	0	0	0	0		
35DIMETHYLPHENOL	122.1664	-95	0	0	1	0		
35DITBUTYLPHENOL	206.3272	-125	0	20	3	0		
3CLC6H4NHNCCNCOOET	251.6719	-91	0	29	6	0		
3ETOCOPYRIDO12APYRIMIDIN4ONH76ME	238.2858	-100	0	20	3	0		
3METHIO4AMINO6ETHYL124TRIAZINE5ONE	186.2312	-116	0	29	2	0		
3METHIO4AMINO6ME124TRIAZINE5ONE	172.2044	-112	0	8	1	0		
3METHOXY4AMINO6IPR124TRIAZINE5ONE	184.1974	-112	0	8	2	0		
3PHENYLAMINO4AMINO6IPR124TRIAZINE5ONE	245.2834	-91	0	29	3	0		
3PROPYL4IPROPYLPHENOL	178.2736	-171	0	0			5	1.07
3PYRIDINEMETHANOLA2PROPYNYLA34DICLPHENYL	292.164	-170	0	26			5	1.47
3PYRIDINEMETHANOLA2PROPYNYLA4FLUOROPHENYL	241.2643	-170	0	26			5	1.48

Table 52

3PYRIDINEMETHANOLA2PROPYNYLA4TOLYL	237.3006	-167	0	29			5	1.06
41HPTERIDINIMINE167TRIMETHYL	189.2194	-95	0	0	0	0		
45DICHLORO2METHOXYPHENOL	193.0292	-95	0	0	2	0		
4ETHYL3PROPYLPHENOL	164.2468	-171	0	0			5	1.3
4METHYLNETHYLHEXAHYDROFLUOREN4AAMINE	229.3644	-110	0	35	2	0		
4OCTANONEGUANYLHYDRAZONE	170.257	-196	0	0			4	1.86
4PTERIDINAMINE12DIHYDRO2IMINE167TRIMETHYL	204.234	-95	0	0	0	0		
5AMINO124DICHLOROPHENYLTETRAZOLE	230.056	-81	0	14	1	0		
5NONANOL	144.2564	-224	0	23			4	1.55
5NONANOL5BUTYL	200.3636	-300	0	23			4	1.89
622DIME5ME13DIOXAN5YLAM24DIAZIRIDTRIAZ	306.3668	-120	0	0	4	0		
62PENHYDROPYRANYL4AM3METHIO124TRIAZINONE	242.2952	-64	0	56	2	0		
6IPROPYL4AMINO3MEAMINO124TRIAZIN5ONE	183.2126	-120	0	0	2	0		
8QUINOLINAMINE6METHOXY	174.2018	-95	0	0	1	0		
8QUINOLINOL6TRIFLUOROMETHYL	213.1587	-120	0	0	1	0		
AAMIDO4METHYLETHYLCINNAMATE	233.2664	-120	0	0	5	0		
AAMIDOMETHYLCINNAMATE	205.2128	-72	0	23	4	0		
ACETANILIDE23DICHLORO5TRIFLUOROMETHYL	272.0537	-120	0	0	2	0		
ACETANILIDE2BROMO35DICHLORO	282.9515	-95	0	0	2	0		
ACETANILIDE2BROMO3TRIFLUOROMETHYL5CHLORO	316.5047	-120	0	0	2	0		
ACETANILIDE2CHLORO3TRIFLUOROMETHYL5BROMO	316.5047	-120	0	0	2	0		
ACETANILIDE35DIMETHYL	163.2188	-95	0	0	2	0		
AJMALINE	326.4376	-97	0	23	3	0		
ANILINE2METHOXY5CHLORO	157.5993	-95	0	0	1	0		
ANILINE2METHOXY5METHYL	137.181	-95	0	0	1	0		
ANILINE35DIBROMO	250.9202	-95	0	0	0	0		
ANILINE35DIIODO	344.9212	-95	0	0	0	0		
ANILINE35DIMETHOXY	153.1804	-95	0	0	2	0		
ANILINE3CHLORO5METHOXY	157.5993	-95	0	0	1	0		
ANTAZONITE	268.348	-171	0	0			5	0.78
APHENYLBUYRAMIDE	163.2188	-157	0	14			4	1.85
ASPARTICACIDDIETHYLESTER	189.2108	-192	0	29			4	1.83
ASPIDINOL	224.256	-122	0	23	6	0		
BAYA6781	210.31186	-125	0	20	2	0		

Table 53

BENZAMIDE23AZETIDINYLTRIAZENE	204.231	-95	0	0	3	0		
BENZAMIDE23METHYL3BUTYLTRIAZENYL	234.3004	-109	0	62	6	0		
BENZAMIDE23METHYL3ETHYLTRIAZENYL	206.2468	-100	0	20	4	0		
BENZAMIDEODICHLOROACETYLAMINO	247.0804	-118	0	2	4	0		
BENZAMIDEOISOPROPYLAMINO	178.2334	-91	0	29	3	0		
BENZENEETHANEAMINE2METHOXY	151.2078	-131	0	14	3	0		
BENZENEETHANEAMINEAHYDROXYNETHYL34DIMETHOXY	225.287	-191	0	5			4	1.82
BENZENEETHANEAMINEAHYDROXYNMETHYL34DIMETHOXY	211.2602	-169	0	2			5	2.34
BENZENEETHANEAMINENSBUTYLAHYDROXY34DIMETHOXY	253.3406	-216	0	5			4	1.82
BENZENEMETHANAMINE34DIMETHOXY	167.2072	-118	0	2	3	0		
BENZENESULFONAMIDE2IBUTYROYLAMINO	242.2922	-110	0	35	4	0		
BENZENESULFONAMIDE2IBUTYROYLAMINO4METHOXY	272.3184	-116	0	29	5	0		
BENZIMIDAZOLE2HYDROXYMETHYL1METHYL	162.1908	-120	0	0	2	0		
BENZOFURAN3AMINE23DIHYDRO5METHYLN1PYRROLIDINYLACETYL	260.3352	-127	0	44	4	0		
BENZOPHENONEHYDRAZONE	196.2512	-90	0	5	2	0		
BENZOTRIAZOLE12HEXANAMIDE	232.2846	-204	0	17			4	1.48
BENZYLALCOHOL2HYDROXY35DICHLORO	193.0292	-118	0	2	3	0		
BENZYLALCOHOL35DIBROMO2HYDROXY	281.9312	-118	0	2	3	0		
BENZYLALCOHOL35DIMETHOXY4HYDROXY	184.1914	-120	0	0	5	0		
BENZYLSULFONAMIDE2METHOXYCARBONYL	229.2502	-116	0	29	4	0		
BETABENZALBUTYRAMIDE	175.2298	-119	0	26	3	0		
BICYCLO310HEXAN3OL4METHYL1ISOPROPYL	154.2516	-120	0	0	2	0		
BUTYLAMINE1PHENYL	149.2352	-148	0	23			4	1.41
C3C1BUTENYL4CHYDROXYMECYCLOPENTENE	152.2358	-171	0	0			5	0.78
CGP39551	237.19186	-207	0	14			4	2.32
COMG1774	237.3412	-212	0	35			4	1.58
CYANAZINE	240.6949	-171	0	0			5	1.28
CYPENAMINE	161.2462	-93	0	2	1	0		
CYPRODINIL	225.2926	-95	0	0	3	0		
DAZADROL	287.7481	-127	0	44	4	0		

Table 54

DECANOICACIDHDRAZIDE	186.2966	-297	0	0			4	1.44
DESETHYLATRAZINE	187.6315	-120	0	0	2	0		
DESISOPROPYLATRAZINE	173.6047	-106	0	14	2	0		
DIACETONEGLUCOSE	260.2864	-45	0	50	2	0		
DIHYDROCODEINE	301.3846	-81	0	14	2	0		
DIHYDROMORPHINE	287.3578	-90	0	5	2	0		
DIMETHIRIMOL2	209.2906	-171	0	0			5	2.36
DIMETHYLGUAIACOL	152.1926	-95	0	0	2	0		
DIMETHYLKETOMALONATEOXIME	161.114	-90	0	5	5	0		
DIMETHYLTARTRATE	178.1414	-171	0	0			4	1.86
DIMETOFRINE	227.2596	-171	0	0			5	2.49
DIPROBUTINE	157.2984	-227	0	20			4	1.47
DIPROPYLHYDANTOIN	184.2376	-191	0	5			4	2.02
DOM-3	209.2876	-125	0	20	4	0		
ECGONINEMETHYLESTER	199.2492	-115	0	5	3	0		
ECTYLUREA	156.184	-120	0	0	4	0		
EFAROXAN	216.2822	-97	0	23	2	0		
ETHANOLAMINENPROPYLN2HYDROXYBUTYL	175.2704	-270	0	2			4	2.27
ETHANOLAMINENTBUTYLN2HYDROXYBUTYL	189.2972	-64	0	56	8	0		
ETHENZAMIDE	165.1914	-91	0	29	3	0		
ETHYLAMINEN2HYDROXYPROPYLN2HYDROXYBUTYL	175.2704	-247	0	0			4	1.86
ETHYLAMINENNBIS2HYDROXYBUTYL	189.2972	-272	0	0			4	2
ETHYLENEGLYCOL12BIS6METHYLPYRID2YL	244.2926	-171	0	0			5	1.31
FENIPENTOL	164.2468	-182	0	14			4	1.99
FLUORENE9HYDROXYMETHYL	196.2482	-120	0	0	2	0		
FR115427	223.317	-54	0	41	1	0		
GLYCEROL13DIETHYLETHER	148.2016	-218	0	29			4	1.74
GUAIETOLIN	212.245	-196	0	0			4	2.59
HEXANOICACID2ACETYLAMINOMETHYLESTER	187.2382	-198	0	23			4	1.8
HEXAPRADOL	283.4126	-249	0	23			4	2.15
HEXYLAMINE1PHENYL	177.2888	-195	0	26			4	1.99
HYDROQUINONE23DIMETHOXY5METHYL	184.1914	-95	0	0	4	0		
HYDROQUINONE26DICHLORO	179.0024	-95	0	0	2	0		
HYDROQUINONE26DIMETHOXY	170.1646	-95	0	0	4	0		

Table 55

HYDROQUINONE2METHYL6BROMO	203.0351	-95	0	0	2	0		
HYDROXYATRAZINE2	197.2394	-119	0	26	4	0		
IMIDAZOLE4CARBOXAMIDE533BISCHLOROETHYLTRIAZINYL	279.1284	-196	0	0		0	4	1.47
INDANOREX	191.2724	-107	0	38	3	0		
ISOLEUCINENACETYLNMETHYLAMINOAMIDE	186.2534	-167	0	29		0	5	0.91
LEUCINEETHYLESTER	159.2278	-176	0	20		0	4	1.77
LUPININE	169.2662	-120	0	0	2	0		
METHAMINAZOLAMIDE	251.2778	-120	0	0	3	0		
METHYLBENZOATE2SULFONAMIDO	215.2234	-109	0	11	3	0		
MILSTEM	209.2906	-95	0	0	6	0		
MISONIDAZOLEFLUORO	219.1721	-219	0	2		0	4	1.94
MORPHOLINE325DIMETHOXYPHENYL	223.2712	-75	0	20	3	0		
N2N2N4N4TETRAMETHYLMELAMINE	182.2278	-95	0	0	2	0		
N2TBUTYLN1BENZENESULFONYLUREA	256.319	-130	0	41	5	0		
NAPHTHALENE1AMINO3CHLORO	177.6329	-112	0	8	0	0		
NBUTYL4CHLOROCINNAMAMIDE	237.7285	-118	0	53	6	0		
NMECARBAMICACIDOCYCLOPENTENYLPHENYLESTER	217.267	-95	0	0	4	0		
NMETHYL2ETHYLPHENYLCARBAMATE	179.2182	-120	0	0	4	0		
NMETHYL2IPROPOXYPHENYLCARBAMATE	209.2444	-120	0	0	5	0		
NMETHYLCARBAMATE3IPROPYL5METHYLPHENYL	207.2718	-120	0	0	4	0		
NMETHYLCARBAMATEOSBUTYLPHENYL	207.2718	-101	0	44	5	0		
NMETHYLCARBAMICACIDOCYCLOPENTYLPHENYLESTER	219.28828	-95		0	4	0		
NNDIETHYLANILINEMHYDROXY	165.2346	-145	0	0		0	4	1.41
NOMETHOXYPHENYL3NPIPERIDINOACETAMIDE	248.3242	-110	0	35	5	0		
NORALPRENOLOL	207.2718	-221	0	0		0	4	1.86
NSC118742	196.2546	-95	0	0	3	0		
OBENZYLOXYBENZAMIDE	227.2622	-119	0	26	4	0		
OCTAHYDROPHENANTHREN4AAMINE9METHYL	215.3376	-87	0	8	0	0		
OPMETHYLBENZYLOXYBENZAMIDE	241.289	-104	0	41	4	0		
OPTUNAL	289.28546	-109	0	11	6	0		
OSDIMENPRPHOSPHORAMIDOTHIOATE	183.20486	-127	0	44	5	0		
OSDIPROPYLPHOSPHORAMIDOTHIOATE	197.23166	-207	0	14		0	4	1.9

Table 56

PENTANOICACID2ACETYLAMINOMETHYLESTER	173.2114	-196	0	0		0	4	2.16
PENTYLAMINEAPHENYL	163.262	-170	0	26		0	5	1.5
PHENAMACIDE	221.2986	-207	0	14		0	4	1.56
PHENOL2ETHYL5METHYL	136.1932	-120	0	0	2	0		
PHENOL2METHOXY4ETHYL	152.1926	-120	0	0	3	0		
PHENOL3DIETHOXYPHOSPHINYL	230.19986	-125	0	20	6	0		
PHENOL3ETHYL4METHYL	136.1932	-120	0	0	2	0		
PHENOL5ETHYL3METHYL	136.1932	-120	0	0	2	0		
PHENYLALANINEMETHYLESTER	179.2182	-171	0	0		0	4	1.89
PHENYLBORONICACID246TRIMETHYL	164.0105	-120	0	0	3	0		
PHENYLPTOLYLCARBINOL	198.264	-122	0	23	3	0		
PHOSPHORICAMIDEDIBUTYLESTER	209.22466	-247	0	0			4	1.7
PHOSPHOROHYDRAZIDICACIDDIPHENYLESTER	264.22006	-78	0	17	5	0		
PICOLINHYDROXAMICACID	166.1792	-95	0	0	3	0		
PILOCARPICACIDETHYLESTER	254.3284	-272	0	0		0	4	1.97
PROCYAZINE	252.7059	-171	0	0			5	1.59
PROPYLAMINEN2HYDROXYPROPYLN2HYDROXYBUTYL	189.2972	-272	0	0			4	1.79
PROPYLAMINENNBIS2HYDROXYBUTYL	203.324	-292	0	5			4	2.11
PTERIDINE4AMINO17DIHYDRO16DIMETHYL7OXO	191.192	-95	0	0	0	0		
PURINE6ETHYLAMINO2METHYL	177.2084	-120	0	0	2	0		
PYRAZINE2AMIDINO56DIMETHYL3METHYLAMINO	179.2242	-90	0	5	2	0		
PYRAZOLO34DPYRIMIDINE46BISMETHYLAMINO	178.1962	-90	0	5	2	0		
PYRAZOLO34DPYRIMIDINE4METHYLAMINO6METHYLTHIO	195.2416	-115	0	5	2	0		
PYRIDINE26DICHLORO4HYDROXY	163.9908	-95	0	0	1	0		
PYRIDINE2HYDROXY56BISMETHIOMETHYL	215.3278	-196	0	0		0	4	1.7
PYRIDINE4AMINO26DICHLORO	163.006	-95	0	0	0	0		
PYRIDINE4HYDROXY26BISMETHOXYCARBONYL	211.1738	-95	0	0	5	0		
PYRIMIDINE12DIHYDRO2IMINO146TRIMETHYL	137.184	-95	0	0	0	0		
PYRIMIDINE1456TETRAHYDRO2AHYDROXYAMETHYLBENZYL	204.2712	-110	0	35	3	0		
PYRIMIDINE24DIAMINO5BENZYL6METHYL	214.2694	-131	0	14	2	0		
PYRIMIDINE24DIAMINO5PCHOROBENZYL6METHYL	248.7145	-113	0	32	2	0		
PYRIMIDINE26DIMETHYL4METHYLAMINO	137.184	-95	0	0	1	0		

Table 57

PYRIMIDINE2AMINO26DIMETHOXY	155.156	-95	0	0	2	0		
PYRIMIDINE2AMINO46BISDIMETHYLAMINO	181.24	-90	0	5	2	0	5	1.86
PYRIMIDINE2AMINO46DIMETHYL	123.1572	-95	0	0	0	0	4	1.91
PYRIMIDINE2AMINO4CHLORO6DIMETHYLAMINO	172.6169	-95	0	0	1	0		
PYRIMIDINE2AMINO4CHLORO6METHYLAMINO	158.5901	-95	0	0	1	0		
PYRIMIDINE2BUTYL4CHLORO6HYDROXY	186.6407	-118	0	53	4	0		
PYRIMIDINE2BUTYLAMINO46DIMETHYL	179.2644	-166	0	5				
PYRIMIDINE2HEXYLAMINO	179.2644	-207	0	14				
PYRIMIDINE2HYDRAZINO4METHOXY6METHYL	154.1712	-95	0	0	2	0		
PYRIMIDINE2HYDROXY46DIMETHYL	124.142	-95	0	0	1	0		
PYRIMIDINE2IPENTYLAMINO46DIMETHYL	193.2912	-166	0	5	3	0	5	2.03
PYRIMIDINE456TRISMETHYLAMINO	167.2132	-90	0	5	1	0		
PYRIMIDINE46DIMETHYL2METHYLAMINO	137.184	-95	0	0	1	0		
PYRIMIDINE4ACETAMIDO5ETHOXYCARBONYL2METHYL	223.231	-103	0	17	5	0		
PYRIMIDINE4AMINO6CHLORO2DIMETHYLAMINO	172.6169	-95	0	0	1	0		
PYRIMIDINE4AMINO6CHLORO2METHYLAMINO	158.5901	-95	0	0	1	0		
PYRIMIDINE4AMINO6METHYL5METHYLAMINO2METHYLTHIO	184.2586	-120	0	0	2	0		
PYRIMIDINE4CHLORO26BISMETHYLAMINO	172.6169	-95	0	0	2	0		
PYRIMIDINE4CHLORO2DIMETHYLAMINO6METHYLAMINO	186.6437	-95	0	0	2	0		
PYRIMIDINE4CHLORO36DIHYDRO6IMINO3METHYL2METHYLAMINO	172.6169	-95	0	0	1	0		
PYRIMIDINE4CHLORO6DIMETHYLAMINO2METHYLAMINO	186.6437	-95	0	0	2	0		
PYRIMIDINE4DIETHOXYMETHYL6HYDROXY	198.2212	-221	0	0	1	0	4	1.44
PYRIMIDINE4HYDROXY26DIMETHYL	124.142	-95	0	0	2	0		
PYRIMIDINE4HYDROXY2METHOXY6METHYL	140.1414	-95	0	0				
PYRIMIDINE4IBUTYL26DIMETHYL	179.2644	-145	0	0			5	2.11
PYRIMIDINE4IPENTYLAMINO26DIMETHYL	193.2912	-166	0	5			5	1.78
PYRIMIDINE5AMINO2CHLORO4METHYL6METHYLAMINO	172.6169	-95	0	0	1	0		
PYRIMIDINE5AMINO2METHYL4METHYLAMINO6METHYLTHIO	184.2586	-120	0	0	2	0		
PYRIMIDINE5BUTYL4HYDROXY6METHYLTHIO	198.2824	-191	0	5	2	0	4	2.02
PYRIMIDINE5CARBAMOYL46DIMETHYL2METHYLAMINO	180.209	-87	0	8	2	0		

Table 58

Name								
PYRIMIDINE5CARBAMOYL4METHYL2METHYLAMINO	166.1822	-95	0	0	2	0		
PYRIMIDINE6CHLORO12DIHYDRO2IMINO1METHYL4METHYLAMINO	172.6169	-95	0	14	1	0		
QUINAZOLINE2TBUTYL34DIHYDRO4HYDROXY	204.2712	-106	0	20	2	0		
QUINOLINETRANSDECAHYDRO4CHLORO2METHYL	187.7119	-75	0	20	0	0		
RA81914	316.3992	-100	0	20	4	0		
RAUWOLFINE	314.4266	-94	0	26	3	0		
SAMPIRTINE	217.2453	-125	0	20	2	0		
SECBUMETON	225.293	-166	0	5		0	5	1.5
SELAGINE	242.32	-81	0	14	0	0		
SIMAZINE2CYCLOBUTYLAMINEANALOG	227.6961	-140	0	5		0	5	1.1
SIMAZINE2CYCLOPROPYLAMINEANALOG	213.6693	-116	0	29	4	0		
SIMAZINE2CYCLOPROPYLMETHYLAMINEANALOG	227.6961	-171	0	0		0	5	1.32
STRIAZINE2METHYLAMINO6TRICHLOROMETHYL	241.5071	-120	0	0	1	0		
SYMTRIAZINE2DIETHYLAMINO4IPROPYLAMINO6METHOXY	239.3198	-171	0	0		0	5	1.18
TARTARICACIDDIETHYLESTER	206.195	-201	0	20		0	4	1.81
TERBUMETON	225.293	-128	0	17	5	0		
TETRAHYDROPYRAN24DIONE31ETHOXYIMINOBUTYL66SPIRO4METHYLCYCLOHEXYL	309.4046	-130	0	41	6	0		
THIAZOLO54DPYRIMIDINE7AMINO5BUTYL	208.2806	-115	0	56	3	0		
TIQUINAMIDE	206.305	-120	0	0	1	0		
TOLDIMFOS	197.17306	-72	0	23	1	0		
TRIBUTYLTINHYDROXIDE2	307.0426	-124	0	47	10	1		
TRIMETHYLCITRATE	234.2054	-192	0	29		0	4	1.56
TYROSINEMETHYLESTER	195.2176	-171	0	0		0	4	1.46
UREA1ETHYL1OANISYL	194.2328	-120	0	0	4	0		
UREAIPROPYL1MTOLYL	192.2602	-131	0	14	4	0		
UREAIPROPYL1PHENYL	178.2334	-116	0	29	4	0		

EXAMPLE 7
Decrease of activity by LBD deletion mutant

The use of a Nor1 gene that completely lacked the LBD region in the Mammalian Two Hybrid reporter system led to a remarkable suppression of the transcriptional activity due to prostaglandin A_2 (Fig. 7). Therefore, prostaglandin A_2 was implicated to function by actually acting on the LBD region of the nuclear receptor.

EXAMPLE 8
Demonstration of the binding of PGA derivatives to Nor1 using BIAcor

In order to indisputably prove the ligand binding activity of the PGA derivatives to Nor1 demonstrated by the Mammalian Two Hybrid reporter system, GST-LBD of Nor1 was expressed in *E. coli* and purified. Through the comparison with GST, the sign of the binding of PGA1 and PGA2 to the LBD of Nor1 was detected using BIAcor S51 (Fig. 8). The negative control compound, 13,14-dihydro-15-keto-PGA2, did not show any activity in the reporter system and did not bind to LBD.

EXAMPLE 9

Enhancement of a gene that is suggested to have such apoptotic character in peripheral blood eosinophils in the remission stage due to therapy of atopic dermatitis corresponds well to the decrease in the number of peripheral blood eosinophils. Therefore, the expression induction of the gene is highly likely to correspond to therapeutic effects. Thus, the type of stimulation that causes expression of this gene in eosinophils was investigated *in vitro*.

A large amount of peripheral blood eosinophils were collected from healthy subjects, and cultured in suspension in a petri dish while suppressing their activation. The activation of the eosinophils via the stimulation with cytokines, such as IL-5 and IL-4, did not lead to the induction of Nor1. In contrast, when apoptosis of the cells was induced with anti-CD30 antibody, Nor1 was revealed to be dramatically induced in the cultured peripheral blood eosinophils during 1 to 3 hr treatment (Table 59, fig. 9). The anti-CD30 antibody that has an agonistic activity against eosinophilic CD30 has recently been receiving attention, because it

induces apoptosis of eosinophils via a specific intracellular pathway and may serve as a therapeutic agent for asthma. Table 59 below summarizes the apoptosis induction of human peripheral blood eosinophils.

5

Table 59

Apoptosis induction of human peripheral blood eosinophils

		Annexin V-positive cells (%)
Fresh		4.0
Control	1 hr	2.30
Anti-CD30 antibody		9.20
Anti-Fas antibody		5.20
Control	3 hr	4.50
Anti-CD30 antibody		20.00
Anti-Fas antibody		13.80
Control	24 hr	11.70
Anti-CD30 antibody		63.00
Anti-Fas antibody		31.20

Although the reaction was slower than with anti-CD30 antibody,
10 apoptosis was induced with anti-Fas antibody. However, anti-Fas antibody did not induce Nor1. Thus, the apoptosis by anti-CD30 antibody accompanied by induction of Nor1 may occur through an eosinophil-specific apoptosis pathway that is different from the conventional pathway. Such phenomena (apoptosis induction and
15 expression induction of Nor1) were similarly observed when AML14.3D10, an eosinophil-specific cell line, was treated with anti-CD30 antibody (Figs. 10 and 11).

It is very likely that such promotion of a pathway that specifically leads eosinophils to cell death through the enhancement
20 of the function of Nor1 leads to the treatment of not only asthma but also various allergic diseases including atopic dermatitis investigated by the present inventors. An example of the therapeutic strategy intended by the present inventors is shown in Fig. 12.

EXAMPLE 10

Establishment of transgenic (TG) mice

A TG mouse in which the human Nor1 gene had been introduced under the actin promoter was successfully established (Fig. 13). The body weight of this mouse was only half of the wild type, and atrophy of the thymus and pancreas was observed. Pancreatic cells decreased in number and their activity declined. Such TG mice can be used as animal models for Nor1-mediated allergic disease analysis.